CFTRI-MYSORE
450
Micromethods of

50 ① micromethods
, balance ③ weighing ⑥
④ laboratory utensils
⑤ standard solutions
⑥ elementary analysis
⑦ nitrogen determination
⑧ carbon ⑨ hydrogen
⑩ halogens ⑪ sulfur ⑫ phospho
-rus ⑬ molecular weight

261	27 DEC 1957	12 DEC 1957
43	15·2·58	
151.	28/11/60	1/11
D 93	11/1/62	29/12/61
220	8/6	31/5/62
346.	4/12	2/12
10.	16/12	27/12.
4/6	18/6	
/6		
	24/7/69	24·7·69

34

Micromethods of
QUANTITATIVE ORGANIC ANALYSIS

By

JOSEPH B. NIEDERL, Ph.D.
Professor of Chemistry, New York University
Graduate School and Washington Square College

and

VICTOR NIEDERL
Teaching Fellow, New York University
Washington Square College

Second Edition

NEW YORK
JOHN WILEY & SONS, INC.
LONDON: CHAPMAN & HALL, LIMITED

E1,5:3;T-R
N42
450.

COPYRIGHT, 1938, 1942, BY
JOSEPH B. NIEDERL
AND
VICTOR NIEDERL

All Rights Reserved

This book or any part thereof must not be reproduced in any form without the written permission of the publisher.

SECOND EDITION

Fourth Printing, September, 1948

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, * MYSORE. *
LIBRARY

CFTRI-MYSORE
450
Micromethods of.

PRINTED IN THE UNITED STATES OF AMERICA

PREFACE TO THE SECOND EDITION

The reviews given the First Edition of this laboratory manual have encouraged the authors not only to retain and continue the mode of presentation as inaugurated therein but also to enlarge and widen the scope of the material presented. Thus, new developments in the authors' laboratory, which include the use of ordinary analytical balances in quantitative organic microanalysis, new molecular-weight determinations, and numerous other improvements and simplifications, as well as the latest and more important contributions by others, have been included in the present edition.

In this laboratory manual, the methods presented have been strictly separated from historical and discursive matters through the introduction of separate headings "remarks" and "literature" at the end of every chapter. This separation, which frees the actual working procedures from distractive matters, has the further advantage of presenting the entire development and literature of a given method in cumulative and easily surveyable form.

In the present edition, which is being published at the *thirtieth anniversary* of the introduction of practical quantitative micromethods into organic chemistry by the late Nobel laureate F. Pregl, teacher of the senior author, the literature cited comprises well above a thousand references by more than eight hundred authors, a fact which most expressively illustrates the interest and rapid progress made in this branch of science.

The authors wish to express their sincere thanks and appreciation to their colleagues, coworkers, students, and visiting professors for the many constructive suggestions which have been incorporated in this book.

Joseph B. Niederl
Victor Niederl

New York City
October, 1941

CONTENTS

INTRODUCTION 1

THE BALANCE 6
  Required precision 6
  The microanalytical balance 7
    Description, 7. Installation, 7. Cleaning, 9.
  The ordinary analytical balance 11
  Remarks 12
  Literature 12

WEIGHING 15
  Microanalytical balance 15
    Zero reading, 16. Sensitivity, 17. Precision, 17. Sensitivity correction table, 21. Adjustment of sensitivity, 21.
  Ordinary analytical balance 22
    Zero reading, 23. Sensitivity, 23. Precision, 23.
  Actual weighing, 26. Microanalytical balance, 27. Ordinary analytical balance, 28. Calibration of weights, 28. Preparation of counterpoises, 30.
  Literature 31

WEIGHING EQUIPMENT 33
  Forceps, 33. Spatula, 33. Probing wire, 33. Camel's-hair brush, 33. Desiccator, 34. Platinum wire, 34. Metal tares, 34. Tare bottles, 34. Rack, 34. Chamois, flannel, cotton, 35. Brushes, 35.
  Literature 35

LABORATORY UTENSILS 36
  Microburners, 36. Gas burners, 36. Drying blocks, 36. Hand centrifuge, 37. Pellet press, 37. Glass-cutting devices, 38. Capillary pipets, 38. Precision pipets, 38. Centrifuge cones, 39. Wash bottles, 39. Miscellaneous, 40.
  Literature 40

PREPARATION AND WEIGHING OF A SAMPLE FOR ANALYSIS 42
  General directions, 42. Sampling, 42. Weighing of the sample, 42. Weighing boats, 42. Weighing tube, 44. Weighing cups and bottles, 45. Capillaries for various wet combustion methods, 45. Capillaries for the vaporimetric molecular-weight determination, 46. Weighing pipets, 46.

Hygroscopic material 48
Literature 49

STANDARD SOLUTIONS 51
Principle 51
Apparatus, 51. Microburet, 51. Buret stand, 52. Dropping bottles, 53. Titration flask, 53. Steaming apparatus, 53.
Reagents, 53. Potassium biiodate, 53. Sodium carbonate, 54.
Indicator solutions, 54. Phenolphthalein, 54. Methyl red, 54. Starch solution, 54.
0.01 *N* Potassium biiodate solution 54
0.01 *N* Hydrochloric acid solution 55
0.01 *N* Sodium hydroxide solution 56
0.01 *N* Sodium thiosulfate solution 56
Remarks, 57. Standard solutions, 57. Indicators, 57. Microburets, 58.
Literature 59

ELEMENTARY ANALYSIS 62
I. DETERMINATION OF METALS 62
Principle 62
Apparatus, 62. Micromuffle, 62. Platinum cylinder, 63.
Reagents, 63. Sulfuric acid, 63. Nitric acid, 63.
Procedure, 63. Calculation, 64.
Remarks 64
Literature 65
II. DETERMINATION OF NEUTRALIZATION EQUIVALENT 66
Principle 66
Apparatus 66
Reagents, 66. Neutral alcohol solution, 66.
Procedure, 67. Calculation, 67.
Remarks 68
Literature 68
III. VOLUMETRIC DETERMINATION OF AMINOID NITROGEN 69
Principle 69
Apparatus, 69. Distillation apparatus, 69. Digestion oven, 70. Digestion flask, 71. Titration equipment, 71.
Reagents, 71. Perhydrol, 71. Catalyzers, 71.
Procedure, 72. Digestion, 72. Distillation, 73. Titration, 74. Calculation, 74.
Remarks 75
Literature 77
IV. GASOMETRIC DETERMINATION OF NITROGEN 79
Principle 79
Apparatus, 79. Kipp generators, 79. Preparation of Kipp generators, 81. Microbubbles, 82. Gasometer, 82. Combustion tube, 83.

Heating unit, 84. Nitrometer, 85. Calibration table, 87. Introduction funnel, 88.
Reagents, 88. Marble, 88. Copper oxide, 88.
Procedure, 89. Introduction of temporary filling and sample, 89. Preparation for the combustion, 91. First combustion, 92. Second combustion and sweeping out, 92. Determination of volume of nitrogen, 93. Determination of the air and adsorption error, 94. Calculation, 95.
Remarks, 95. Dry ice carbon dioxide generator, 97.
Literature 99

V. CARBON AND HYDROGEN DETERMINATION 101
Principle 101
Apparatus, 101. Oxygen tank, 101. Pressure regulator, 101. Preheater, 103. Bubble counter and U-tube, 104. Heating unit, 106. Heating mortar, 106. Combustion tube, 107. Combustion-tube fillings, 107. Simple filling, 107. Simple band filling, 107. Combination filling, 107. Combination band filling, 107. Filling the combustion tube, 108. Conditioning the combustion tube, 110. Absorption tubes, 111. Water absorption tube, 112. Carbon dioxide absorption tube, 113. Counterpoising the absorption tubes, 113. Drying tube and safety tube, 114. Mariotte flask, 114. Aspirator, 115. Miscellaneous, 116.
Assembling the combustion train 118
Reagents, 121. Anhydrone, 121. Ascarite, 121. Copper oxide, 122. Lead peroxide, 122.
Testing the apparatus, 123. Wiping and weighing the absorption tubes, 123. Blank test on the aspirator, 125. Blank combustion tests, 126.
Actual analysis, 128. Preparation of the sample, 129. Combustion, 129. Attaching the absorption tubes, 129. Combustion of sample, 129. Removal of absorption tubes, 131. Calculation, 132.
Remarks, 132. Dry combustion method, 132. Wet combustion methods, 139. Gas volumetric and manometric methods, 140. Semi-micro dry combustion methods, 145.
Literature 146

VI. DETERMINATION OF HALOGENS 151
Gravimetric Methods 151
Wet Combustion (Carius) Method 151
Principle 151
Apparatus, 151. Furnace, 151. Pressure tubes, 151. Filter tubes, 152. Filtration apparatus, 154.
Reagents, 154. Concentrated nitric acid, 154. Silver nitrate, 154.
Procedure, 154. Preparation and weighing of the sample, 154. Filling the pressure tube, 155. Sealing the pressure tube, 155. Digestion, 156. Opening the pressure tube, 156. Filtering the precipitate, 157. Dissolving the precipitate, 158. Calculation, 160.
Dry Combustion Method 160
Principle 160

Apparatus, 160. Oxygen tank and gas wash bottle, 160. Combustion-spiral tube, 160. Combustion stand, 160. Platinum contacts, 160. Precipitation tubes, 160. Filter tubes, filtration apparatus, wash bottles, 161.

Reagents, 161. Sodium bisulfite solution, 161.

Procedure, 162. Preparation and filling of the combustion tube, 162. Combustion, 162. Precipitation and filtration, 163. Iodine, 164. Calculation, 164.

Fusion Method 165

Principle 165

Apparatus, 165. Bomb, 165. Precipitation tubes, filter tubes, filtration apparatus, 165.

Reagents, 166. Sodium peroxide, 166.

Procedure, 166. Mixing, 166. Fusion, 166. Precipitation and filtration, 166. Chlorine, 167. Bromine and iodine, 167. Calculation, 167.

Titrimetric Methods 168

Chlorine and Bromine 168

Principle 168

Apparatus, 168. Oxygen tank and gas wash bottle, 168. Combustion apparatus, 168. Heating block, 169. Titration equipment, 169.

Reagents, 169. Potassium dichromate, 169. Silver dichromate, 169.

Procedure, 170. Preparation and filling of the apparatus, 170. Heating and titration, 171. Calculation, 172.

Iodine 172

Principle 172

Apparatus, 172. Oxygen tank and gas wash bottle, 172. Combustion tube, 172. Combustion stand, 172. Platinum contacts, 172. Precipitation tubes, 172. Titration equipment, 172.

Reagents, 173. Sodium acetate solutions, 173. Bromine, 173.

Procedure, 173. Combustion, 173. Transfer and titration, 174. Calculation, 175.

Remarks, 175. Reviews and reports, 175. Wet combustion methods, 175. Dry combustion methods, 177. Hydrogenation method, 177. Fusion method, 177. Calcination method, 177. Ionizable halogen, 178. Simultaneous determination of chlorine and bromine, 178. Special iodine methods, 178. Filter tube improvements and substitutions, 178.

Literature 179

VII. DETERMINATION OF SULFUR 182

Gravimetric Wet Combustion (Carius) Method 182

Principle 182

Apparatus, 182. Furnace, pressure tubes, 182. Filter funnel, 182. Evaporation apparatus, 182. Porcelain crucible and immersion filter, 183. Suction flask, 184.

Reagents, 185. Barium chloride, 185.

Procedure, 185. Preparation and weighing of the sample, 185. Filling of the pressure tube, 185. Sealing the pressure tube, 185. Di-

gestion process, 185. Opening the pressure tube, 185. Transfer of the reaction product, 185. Evaporation of the sulfate solution, 186. Transfer of the sulfate, 186. Precipitation, 186. Filtration, 186. Drying and ignition, 187. Calculation, 188.
Titrimetric Dry Combustion Method 188
Principle 188
Apparatus, 188. Oxygen tank, gas wash bottle, combustion-spiral tube, combustion stand, and platinum contacts, 188. Titration equipment, 188.
Reagents, 188. Hydrogen peroxide solution, 188.
Procedure, 188. Combustion, 188. Titration, 189. Calculation, 190.
Remarks, 190. Wet combustion methods, 190. Dry combustion methods, 191. Fusion, 193. Filtration, 193. Titration, 195.
Literature 197
VIII. DETERMINATION OF PHOSPHORUS 199
Principle 199
Apparatus 199
Reagents, 200. Molybdate solution, 200. Nitric-sulfuric acid mixture, 200.
Procedure, 200. Wet combustion, 200. Fusions, 201. Precipitation, 201. Filtration, 202. Calculation, 202.
Remarks 202
Literature 204
IX. OTHER ELEMENTS 205
Arsenic, 205. Boron, 206. Copper, 206. Mercury, 206. Oxygen, 207. Selenium, 208. Silver, 208.
Literature 208

DETERMINATION OF THE MOLECULAR WEIGHT 210
I. EBULLIOSCOPIC METHODS 210
Principle 210
Apparatus, 210. Pregl's apparatus, 210. Rieche's apparatus, 211. Sucharda-Bobranski-Schmitt apparatus, 212. Precision pipets, 213.
Solvents 213
Procedure, 213. Preparation of the sample, 213. Preparation of the apparatus, 214. Pregl and Rieche apparatus, 214. Sucharda-Bobranski-Schmitt apparatus, 214. Calculation, 215.
Remarks 215
Literature 216
II. CRYOSCOPIC METHOD 217
Principle 217
Apparatus 217
Solvents, 217. Camphor, 217.
Procedure, 218. Calculation, 219.
Remarks 219
Literature 220

III. VAPORIMETRIC METHOD 221
Principle 221
Apparatus, 221. For low-boiling substances, 221. For high-boiling substances, 221.
Procedure, 223. Low-temperature apparatus, 223. Preparation of the sample, 223. Heating, 224. High-temperature apparatus, 224. Preparation of the sample, 224. Filling the apparatus, 225. Heating, 226. Cleaning the apparatus, 226. Correction for heat expansion, 226. Calculation, 227.
Remarks 227
Literature 228

IV. ISOTHERMIC METHOD 230
Principle 230
Apparatus, 230. Microscope, 230. Glass plate, 230. Capillaries, 231. Colored glass thread, 231. Desiccator tubes, 231. Glass wool, 231. Adhesive tape, 231. Volumetric flasks, 231. Pipets, 231. Hand centrifuge, 231. Vacuum pump, 231.
Reagents, 231. Standard solutions, 231.
Procedure, 232. Preparation of the sample solution, 232. Preparation of the capillaries, 233. Filling the desiccator tube, 233. Mounting the desiccator tubes, 233. Method of reading, 234. Interpretation of readings, 236. Calculation, 236.
Remarks 237
Literature 238

STRUCTURE ANALYSIS 239

I. DETERMINATION OF ALKOXYL AND ALKIMIDE GROUPS 239
Alkoxyl Groups 239
Principle 239
Apparatus, 239. Alkoxyl apparatus, 239. Kipp generator, 241. Flasks, 241. Buret, 241.
Reagents, 241. Hydriodic acid, 241. Washer solution, 241.
Procedure, 242. Preparation of the sample, 242. Introduction of the sample and solubility tests, 242. Preparation of the apparatus and heating, 242. Titration, 243. Calculation, 244.
Alkimide Groups 244
Principle 244
Apparatus 244
Reagents, 245. Gold chloride, 245. Ammonium iodide, 245.
Procedure, 246. Preparation of the sample and apparatus, 246. Heating, 246. Titration, 246. Calculation, 246.
Remarks, 247. *O*-Alkyl, 247. Iodometric methods, 247. Gravimetric methods, 248. *S*-Alkyl, 249. *N*-Alkyl, 249. Hydriodic acid, 249. Other methods, 250.
Literature 250

II. DETERMINATION OF ACYL GROUPS 252
Alkalimetric Method 252

Principle 252
Apparatus, 252. Bubble counter and U-tube, 252. Hydrolysis apparatus, 252. Water bath, 253. Pipets, 253. Titration equipment, 253.
Reagents, 254. *p*-Toluene sulfonic acid, 254.
Procedure, 254. Solubility tests, 254. Preparation of the sample, 254. Preparation of the apparatus, 255. Hydrolysis, 255. Distillation, 255. Titration, 256. Calculation, 256.
Iodometric Method 257
Principle 257
Apparatus, 257. Acetyl apparatus, 257. Titration equipment, 257.
Reagents, 257. 0.01 *N* iodine solution, 257.
Procedure, 258. Preparation of the apparatus, 258. Charging of the distilling flask, 259. Heating, 259. Transfer of the distillate and titration, 260. Correction for sulfur dioxide, 260. Calculation, 261.
Remarks 261
Literature 262

III. DETERMINATION OF GROUPS REACTIVE TO GRIGNARD REAGENT . . . 263
Principle 263
Apparatus, 264. Methane generator, 264. Methanometer, 264.
Reagents, 265. Nitrogen, 265. Solvents, 266. Grignard reagent, 266.
Filling the apparatus, 267. Determination of the blank, 267.
Procedure, 269. Preparation of the apparatus, 269. Introduction of the sample, 269. Generation of methane, 269. Addition of aniline, 270. Calculation, 270.
Remarks 270
Literature 271

IV. OTHER METHODS 273
Amines, 273. Electrometric and photoelectric methods, 273. Hydrogenation methods, 273. Isopropylidine groups, 273. *C*-Methyl groups, 274. Optical rotation, 274.
Literature 274

APPENDIX 277
THE TEACHING OF QUANTITATIVE ORGANIC MICROANALYSIS 277
INSTALLATION OF A LABORATORY FOR QUANTITATIVE ORGANIC MICROANALYSIS 283
QUALITATIVE ORGANIC MICROANALYSIS 290
CALCULATIONS 296
NITROGEN REDUCTION TABLES 301
LOG TABLES 312

AUTHOR INDEX 331

SUBJECT INDEX 341

INTRODUCTION

One of the noteworthy advances in chemistry has been the introduction of methods and technics permitting chemical operations with smaller amounts of substances than commonly used, a fact well recognized in chemical research, both organic and inorganic. In certain branches of chemistry the application of micromethods has become a necessity, particularly in the field of hormones and vitamins. Numerous successful applications of microtechnic to qualitative organic chemical problems of toxicological, clinical, and pathological nature were reported in the chemical literature as early as the second part of the last century. One of the earliest contributions to this branch of science was made in the United States in 1867, in which year the monograph *Microchemistry of Poisons* by T. G. Wormley [39] was published.

Among the first successful attempts in quantitative organic analysis to decrease the amount of the sample from decigrams to centigrams was the molecular-weight method of G. Barger,* [2] published in 1904. Quantitative inorganic microanalyses appear to have been first performed in 1903 by W. Nernst and E. H. Riesenfeld,[14, 15] when atomic-weight determinations on inorganic samples weighing 1 mg. and less were described. An empirically calibrated microbalance was used. Subsequently, in 1909, F. Emich † and co-workers reported the first quantitative milligram (micro) processes with pure organic compounds,[9] such as a micro-Carius procedure for sulfur ‡ and halogen,§ and in 1911 [25] a micro-Kjeldahl method,‖ using the microbalance of W. Nernst.[14, 15] The substitution of this microbalance by the Kuhlmann microchemical balance in quantitative microanalysis was recommended by J. Donau [5] in 1911.

Using this balance, F. Pregl ¶ succeeded in the same year [28] in revolutionizing most of the existing macrochemical methods in quantitative organic analysis of pure organic compounds by the introduction of equivalent micromethods. These procedures permitted determinations with samples amounting to a few milligrams and involved not only manipulative microtechnic, but also complete co-ordination of apparative designs to such processes.[28–33] In the subsequent and widespread use of the Pregl microanalytical methods for pure organic substances, the saving in material and time—features which are usually associated with and demanded of microchemical procedures—were decisive contributing factors. Their practicability was an additional advantage which gradually led to a complete replacement of the corresponding macromethods used heretofore.[16] This general adaptation of the milligram processes brought about the introduction of these methods both in industrial organic research laboratories and in the teaching curricula of institutions offering advanced chemical instruction.

---

* 31-mg. sample.

† Died January 22, 1940.

‡ 0.3- to 1-mg. samples.

§ 1- to 3-mg. samples.

‖ 0.4- to 2-mg. samples.

¶ Died December 13, 1930.

Certain difficulties arose in the transfer in 1925 of the microprocedures from the ideal location and laboratory arrangements as provided in F. Pregl's own institute at the University of Graz, Austria, to the authors' laboratory at New York University, Washington Square College, New York, N. Y., U. S. A. With the subsequent systematic teaching of these methods by the authors [16a] within the set framework of a graduate laboratory course (Chemistry 202) further difficulties had to be overcome, which necessitated the changes in the above microprocedures as presented in the first [19] and continued in the present edition of this book.*

In the present edition the chapters on balances and weighing have been enlarged to include the use of ordinary analytical balances of proper sensitivity and precision.[17] Additional paragraphs treating the calibration of weights, the determination of the zero reading, and the determination of the sensitivity and precision of the microanalytical as well as the ordinary analytical balance have also been included in the present volume.

To the changes in the carbon and hydrogen determination [3, 13, 16c, 18, 19, 28–33, 38] as given in the first edition, which included a practical preheater, the side-arm combustion tube, combustion in oxygen, new absorption reagents, as well as an aspirator for the absorption tubes, have been added several types of combustion-tube fillings.[19a] Wet combustion methods for the determination of carbon are also discussed, and the manometric method of D. D. Van Slyke and J. Folch [37] has been described in detail in the present edition.

---

* Reviews of the first edition: *J. Am. Chem. Soc.*, **61**, 2985 (1939); *Ind. Eng. Chem., News Ed.*, **16**, 241 (1938); *Analyst*, **63**, 467 (1938); *Microchemie*, **24**, 237 (1938); *Z. anal. Chem.*, **113**, 293 (1938); *J. Indian Chem. Soc.*, **15**, 404 (1938); *J. Chem. Soc., Japan*, **59**, 966 (1938); etc.

The volumetric determination of aminoid nitrogen (Kjeldahl method) remained unchanged from the original Pregl procedure [30–34] also in the present edition. The improvements in the gasometric determination of nitrogen (Dumas method), which included a double Kipp generator and the introduction of a gasometer, [20, 21, 36] by means of which the amount of carbon dioxide used in the combustion and sweeping-out process is measured, have been retained unchanged from the first edition.

The determinations of halogen and sulfur have been improved,[27] while the wet combustion method of M. K. Zacherl and H. G. Krainick [40] and the bomb method of A. Elek and D. W. Hill,[8] which were introduced in the first edition, have been retained in the present volume. The same is true of the application of F. Emich's immersion filter,[10] as recommended by W. Saschek [34] for the filtration of barium sulfate. The use of a platinum cylinder in the determination of metals, as suggested by H. K. Alber [1] and H. I. Coombs,[4] is also retained.

The standard solutions have now been unified in a single chapter, and with the introduction of the 0.01 N potassium biiodate solution, which takes the place of the 0.01 N hydrochloric acid solution, the preparation and standardization of the 0.01 N solutions used in titrimetric procedures have been simplified. To the ebullioscopic, cryoscopic, and vaporimetric molecular-weight methods has been added an isothermic method.[16b, 22–25] Liberal time estimates have been given for all the more important determinations in order to facilitate the planning of a day's working or teaching schedule.

The structure analytical methods include the determination of active hydrogen by A. Soltys,[35] the acyl determination of R. Kuhn and H. Roth,[12] the alkoxyl and acetyl groups determinations of A. Elek [6] and A. Elek and R. A. Harte,[7] respectively, and the modified alkimide group determination of A. Friedrich.[11]

The literature has been brought up to 1941. In the Appendix are chapters pertaining to qualitative organic analysis,[16d] to the teaching of quantitative organic elementary micromethods,[16a] and to the installation of a laboratory for quantitative organic microanalysis in colleges, research institutions, and organic research laboratories in industry.

LITERATURE

1. Alber, H. K., *Mikrochemie*, **18**, 95 (1935).
2. Barger, G., *J. Chem. Soc.*, **85**, 309 (1904).
3. Boetius, M., "Über die Fehlerquellen bei der mikroanalytischen Bestimmung des Kohlen- und Wasserstoffes nach der Methode von Fritz Pregl," Verlag Chemie, Berlin, 1931.

4. Coombs, H. I., *Biochem. J.*, **21**, 404 (1927).
5. Donau, J., *Monatsh.*, **32**, 35 (1911).
6. Elek, A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 174 (1939).
7. Elek, A., and Harte, R. A., *Ind. Eng. Chem., Anal. Ed.*, **8**, 267 (1936).
8. Elek, A., and Hill, D. W., *J. Am. Chem. Soc.*, **55**, 2550, 3479 (1933).
9. Emich, F., and Donau, J., *Monatsh.*, **30**, 753 (1909).
10. Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York, 1932, pp. 68–75.
11. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933.
12. Kuhn, R., and Roth, H., *Ber.*, **66**, 1274 (1933).
13. Lindner, J., "Mikro-massanalytische Bestimmung des Kohlenstoffes and Wasserstoffes mit grundlegender Behandlung der Fehlerquellen in der Elementaranalyse," Verlag Chemie, Berlin, 1935.
14. Nernst, W., *Göttinger Nachrichten*, 1903, pp. 75–82.
15. Nernst, W., and Riesenfeld, E. H., *Ber.*, **36**, 2086 (1903).
16. Niederl, J. B., *Ind. Eng. Chem., Anal. Ed.*, **7**, 214 (1935); (*a*) *J. Chem. Education*, **13**, 254 (1936); (*b*) *Z. anal. Chem.*, **77**, 169 (1929); (*c*) *Z. anal. Chem.*, **89**, 62 (1932); (*d*) "Semi-Micro Qualitative Organic Analysis," New York University, New York, N. Y., 1941.
17. Niederl, J. B., Niederl, V., Nagel, R. H., and Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).
18. Niederl, J. B., and Roth, R. T., *Ind. Eng. Chem., Anal. Ed.*, **6**, 272 (1934).
19. Niederl, J. B., and Niederl, V., "Micromethods of Quantitative Organic Elementary Analysis," J. Wiley & Sons, New York, N. Y., 1938; (*a*) *Microchemie-Microchim. Acta*, **26**, 28 (1939).
20. Niederl, J. B., and Saschek, W., *Mikrochemie*, **11**, 237 (1932).
21. Niederl, J. B., Trautz, O., and Saschek, W., *Mikrochemie, Emich Festschrift*, 1930, p. 219.
22. Niederl, J. B., and Routh, I., *Mikrochemie*, **11**, 251 (1932).
23. Niederl, J. B., Trautz, O., and Plentl, A., *Ind. Eng. Chem., Anal. Ed.*, **8**, 252 (1936).
24. Niederl, J. B., and Levy, A. M., *Science*, **92**, 225 (1940).
25. Niederl, J. B., and Schmitt, R. B., *Jesuit Science Bull.*, **18**, 88 (1940).
26. Niederl, J. B., Niederl, V., and Eitingon, M., *Mikrochemie-Mikrochim. Acta*, **25**, 143 (1938).
27. Niederl, J. B., Baum, H., McCoy, J. S., and Kuck, J. A., *Ind. Eng. Chem., Anal. Ed.*, **12**, 428 (1940).
28. Pregl, F., *Ber.*, **44**, 553 (1911); Lecture, German Chem. Soc., Feb. 27, 1911.
29. Pregl, F., "Abderhalden's Handbuch der biochemischen Arbeitsmethoden," Vol. V, pp. 1307–1356, Urban und Schwarzenberg, Berlin and Vienna, 1912.
30. Pregl, F., "Die quantitative organische Mikroanalyse," J. Springer, Berlin and Vienna, 1912.
31. Pregl, F., and Roth, H., "Die quantitative organische Mikroanalyse," J. Springer, Berlin, 1935.
32. Pregl, F., and Fyleman, E., "Quantitative Organic Microanalysis," P. Blakiston's Son & Co., Philadelphia, Pa., 1924.
33. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937.
34. Saschek, W., *Ind. Eng. Chem., Anal. Ed.*, **9**, 491 (1937).

35. Soltys, A., *Mikrochemie*, **20**, 107 (1936).
36. Trautz, O., and Niederl, J. B., *Ind. Eng. Chem.*, *Anal. Ed.*, **3**, 151 (1931).
37. Van Slyke, D. D. and Folch, J., *J. Biol. Chem.*, **136**, 509 (1940).
38. Weygand, C., "Quantitative analytische Mikromethoden der organischen Chemie in vergleichender Darstellung," Akademische Verlagsanstalt, Leipzig, 1931.
39. Wormley, T. G., "Microchemistry of Poisons," J. B. Lippincott, Philadelphia, Pa., 1867.
40. Zacherl, M. K., and Krainick, H. G., *Mikrochemie*, **11**, 61 (1932).

THE BALANCE

In principle any balance showing a reproducible sensitivity, or precision, of 0.001 to 0.020 milligram can be used for quantitative organic microanalysis. Two types of balances are employed: a microanalytical balance, or an ordinary analytical balance.

Required Precision

A direct relationship exists between the precision of a balance intended for use in quantitative organic microanalytical procedures [33] and the amount of substance to be used. This relationship is illustrated in the table below.

	Amount of Sample	
Precision of Balance	*Theoretical Minimum*[9]	*Practical Average*[33]
mg.	mg.	mg.
0.001	0.3–0.4	3– 5
0.002	0.6–0.8	4– 6
0.005	1.5–2.0	5– 8
0.010	3.0–4.0	6–10
0.020	6.0–8.0	8–12

For all practical purposes a microanalytical balance should have a minimum precision of ±5 micrograms,* for a load up to 1 gram and of ±10 micrograms for loads above 1 gram. Numerous domestic [2, 7, 35] as well as foreign-made [11, 24, 26, 27, 30, 34, 39, 42] microanalytical balances fulfill these requirements.[1, 12, 13, 19, 21, 23, 29, 38, 41] In keeping with the above it should be borne in mind that the claim often made, but rarely fulfilled, that a microanalytical balance has a precision of ±1 microgram at a maximum load of 20 grams should not be interpreted as an essential requirement for quantitative organic microanalysis.

An ordinary analytical balance intended for use in quantitative organic microanalysis should show a minimum precision of at least 1/50 milligram for loads not exceeding 10 grams.[33, 37a, 49] In actual practice balances which show a precision of 1/100 milligram will be found most useful. Several makes of domestic balances [2, 7, 43] are available, which sometimes are called *semi-micro* or *precision-analytical* balances, that show this precision and also conform to the standard methods of testing of workmanship and satisfactory performance of analytical balances.[1, 14, 22, 28, 40]

---

* One microgram = 0.001 milligram = 1 gamma (γ).

THE MICROANALYTICAL BALANCE

Description

As a typical microanalytical balance, the microchemical balance of Wm. F. Kuhlmann,[24] which was extensively employed by F. Pregl,[37] is herewith described in detail. The balance consists of the balance case, the black glass base, and the weighing mechanism. The last operates through the hollow center column which is mounted on the base and supports the fulcrum. On the fulcrum rests the balance beam, which is a platinum-plated flat sheet of metal shaped somewhat like the letter K. The upper horizontal part of the beam is 7 cm. long and carries the rider scale, which has 101 notches with corresponding numerical marks from 0 to 10 below each tenth notch. The rider scale is so calibrated that the first notch at the end of the beam represents zero, each successive notch 0.1 milligram, and the numerical marks whole milligrams when a 5-mg. rider is used. The center of the beam carries an agate knife edge which faces the agate plate of the fulcrum, and each terminal of the beam is provided with an upright knife edge which contacts the agate plate of the corresponding stirrup. These three agate knife edges must be parallel to each other and in one plane, as well as equidistant from the center. The arrestment rod extends through the hollow center column and carries a horizontal bar provided with three arrestment cups in the front and three arrestment slots in the rear, of which one cup and slot are located in the center and one of each at the left and right terminal.

The horizontal metal part of each stirrup is provided with an agate plate in the center and an agate contact point in the front as well as in the rear which fits into the corresponding agate cup or slot. To prevent the interchange of the stirrups in assembling the balance they are marked with dots which must face forward and coincide with the corresponding marks on the arrestment bar. S-shaped wire hooks, inserted at the acute angle or lowest part of the stirrups, serve for the attachment of the weighing pans. Suitably bent hooks for the support of tubes used in weighing are attached to the suspension wires of the left weighing pan or of both weighing pans.

The pointer, which is 13.5 cm. long, is attached to the center of the beam and extends down to the pointer scale at the base of the center column. The pointer scale is 22 mm. in length and has 12 divisions on each side of the zero line.

The rider used in the balance is made of aluminum, weighs 5 mg., and is shaped like an inverted V, with loops at the apex and terminals. Suitable magnifying devices at the rider and the pointer scale facilitate reading the respective scale. The magnifying device for the pointer scale should be so arranged as to avoid, or at least keep at a minimum, any parallactic errors.

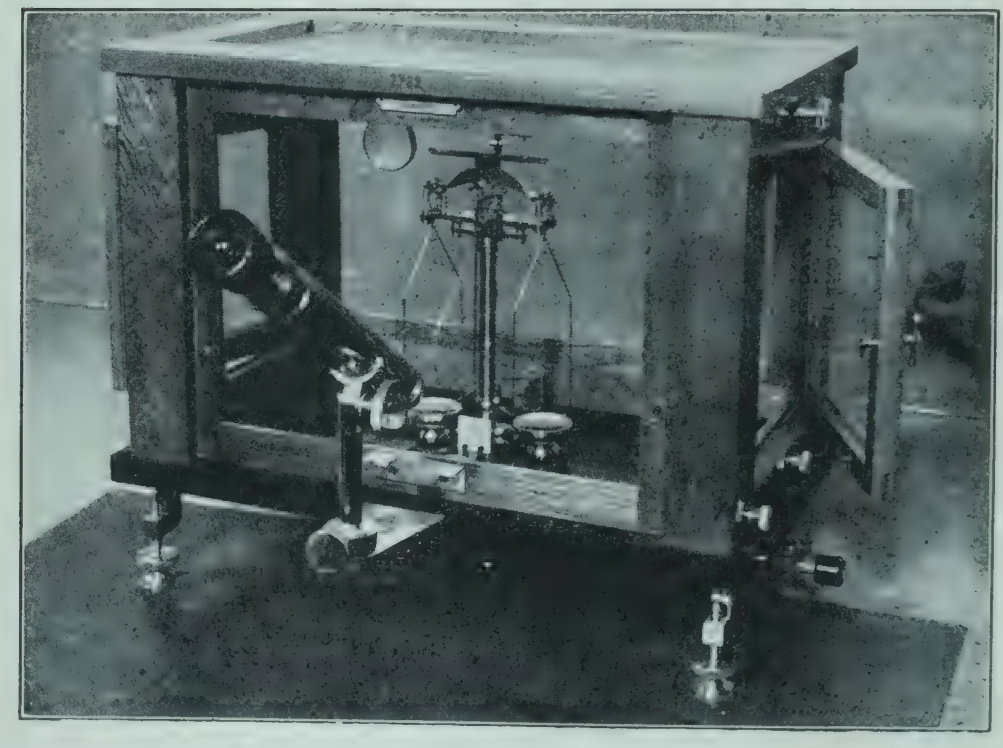


Fig. 1. Typical Microanalytical Balance.

In the Kuhlmann telescope balance ^{24} shown in Fig. 1 a telescope is attached to the base of the front window. The pointer carries a small disk of approximately 3-mm. diameter. This disk, which is in the focus of the telescope, takes the place of the usual pointer scale and swings with the pointer; a stationary hair line in the eyepiece of the telescope serves as the reference line. Owing to the shortness of the swings in this balance, greater constancy and, consequently better reproducibility of weighings are obtainable.

Installation

Since a temperature change of 1 to 2° C. will cause a shift of approximately 10 micrograms [36, 41] in the zero reading, a temperature constancy of ±2° C. is highly desirable. A room should be chosen in which a fairly constant temperature can be maintained while the balance is in use. For the same reason the balance should be placed well away from radiators or heat-radiating devices and should not be exposed to direct sunlight. Artificial light sources should be at least 3 ft. away. Under adverse conditions it would be well to construct a special balance compartment which would allow free circulation of air and yet prevent exposure of the balance to heat or drafts. Satisfactory set-ups for microanalytical balances have been described repeatedly.[3, 4, 6, 10, 12, 13, 21, 23]

Although humidity as a rule does not interfere to any extent with the satisfactory performance of the balance, nevertheless it may cause the beam to stick and prevent the free swinging of the pointer upon release of the arrestment. The ideal humidity is considered to be about 50%. A lower moisture content of the air may permit the accumulation and retention of electrostatic charges on the metal parts of the balance.[37, 46] It is claimed that this condition may be remedied by grounding the balance or by placing a radioactive element in the balance case.[37] The balance requires also a reasonable amount of protection from vibration; usually it is sufficient to provide a solid foundation. A solid wooden table or bench with a top of marble, soapstone, or Transite provides as a rule an adequate support for the balance, although a combination of rubber sheets and lead or stone slabs, may be necessary under adverse conditions. An arrangement for a shock-proof and vibration-free support has been described by W. R. Kirner.[21]

Cleaning and Assembling

The balance, when received from the manufacturer, usually is accompanied by detailed instructions for unpacking and assembling. It is obvious that these instructions must be followed in every detail and that the proper care be exercised in unpacking and assembling the balance, especially in removing or handling the beam, in order to avoid bending the tip of the pointer. The various parts of the new balance are cleaned and assembled just as they are cleaned and assembled periodically after the balance has been in use for some time; therefore, the instructions given below for the care and cleaning of the balance apply also to a new one.

IMPLEMENTS

A sheet of glazed white paper, several thoroughly washed and dried pieces of soft thin chamois, four finger cots of rubber or chamois for thumbs and index fingers, a few round toothpicks, ivory-tipped forceps, a camel's-hair brush washed in acetone, a container with absorbent cotton, a magnifying lens, and a small flashlight are all the necessary tools needed for the cleaning of the balance.

PROCEDURE

First, the table upon which the balance is to be placed is cleaned; then the removable case, if the balance is provided with one, is taken off. In balances having a fixed case, the front window is removed and placed on top of the balance. With the index fingers and thumbs covered with the finger cots, because the metal parts of the balance should never be touched with unprotected fingers, the balance pans are taken off and placed on the glazed white paper which is spread near the balance case; the stirrups are removed next by grasping them by the horizontal metal bar and lifting them off the beam. Then the beam of the balance, being held at the upper half of the pointer, is lifted off the fulcrum and also placed on the paper with the pointer tip upward.

The inside of the balance case is cleaned by wiping it with a chamois and dusting it afterwards with a large camel's-hair brush. The axis and the three cams under the base plate are oiled with a trace of watchmaker's oil. The central agate plate on the fulcrum as well as the agate cups and slots are cleaned with a perfectly dry cotton tuft wound around a toothpick and then thoroughly brushed with a small camel's-hair brush; particular attention must be paid to this operation, because deposits of dust or fibers on the agate plate or in the cups or slots are frequently responsible for a condition known as *sticking* of the balance beam when the arrestment is released. If this sticking cannot be eliminated by ordinary cleaning or wiping of the surfaces of contact, the wiping should be repeated with a cotton tuft moistened with a trace of alcohol or acetone, followed immediately with a thorough wiping with a dry cotton tuft. Finally, the cleaned parts are brushed again and minutely examined with the aid of a small flashlight and a magnifying lens for fibers and dust particles.

The beam is cleaned next. It is held at the pointer somewhat below the horizontal bar, and the three knife edges are wiped also with a clean cotton tuft. The two contact points are treated similarly, and finally the entire beam is brushed free from dust or lint and thoroughly inspected with the aid of a magnifying lens for deposits of dust particles.

Then, with the balance arrested and the hand steadied, the beam is put on the fulcrum by placing the two contact points in the arrestment cup and slot in the front and rear of the arrestment bar.

The agate plate and the two contact points of both stirrups are cleaned with a cotton tuft and brushed, as described above. Then the stirrups are placed in the agate cups of the respective terminals of the arrestment bar so that the markings face the front and coincide with the markings on the arrestment bar. The balance pans and suspension wires are cleaned by wiping them with a chamois and brushing them afterwards. The pan marked with the letter "R" is placed on the S-shaped hook of the right-hand stirrup, and the other pan, marked with the letter "L," is placed on the left-hand stirrup. Then the rider is brushed and placed on the zero notch of the rider scale. Finally, the balance is leveled by turning the adjustment screws at the front support; the plummet in the rear of the center column, or two spirit levels, one of which is placed in front and the other parallel to one of the side doors, are used as level indicators. Then the balance case or the front window is replaced, and about 30 minutes later, during which time the side doors of the balance are left half open, the zero reading is adjusted by turning the adjustment screw on the horizontal rod back of the beam with the aid of the ivory-tipped forceps. The adjustment screw is turned forward if the pointer swings too far to the right and backward if it swings too far to the left of the zero line. However, the balance need not be adjusted to exactly zero; an adjustment which gives a zero reading between zero and ±50 micrograms is quite satisfactory.

THE ORDINARY ANALYTICAL BALANCE

Description

Since the only difference between an analytical balance for macroanalytical and one for microanalytical work is merely an increased precision and sensitivity, there need not be any change in its construction.[7, 33, 37a, 49] For practical purposes and for the sake of uniformity in the weighing procedure, an analytical balance intended for use in microanalytical procedures should possess the following features: The beam should be calibrated and marked from 0 to 10 milligrams,[2] with the zero mark under the first notch at the extreme left. Only notches denoting whole milligrams are needed, and hence the usual subdivisions representing tenths of a milligram may be omitted.[7] A 5-milligram rider is used. The pointer scale should have at least ten divisions on either side of the zero line and should be provided with a magnifying lens.

For continuous proper function of an analytical balance possessing a higher than ordinary precision, it is necessary, in the installation and during the process of cleaning and assembling, to observe the same precautions as for a microanalytical balance.

Remarks

The directions in regard to the performance, assembling, and cleaning of the microanalytical balance have been kept strictly within the scope of a laboratory manual and, therefore, represent the *minimum* requirements such as can be found in the average chemical laboratory. For detailed discussions concerning further refinements, the investigations of F. Emich,[16] E. Schwarz-Bergkampf,[41] L. Ramberg,[38] G. Gorbach,[19] H. Sternberg,[45] A. W. Ainsworth,[1, 29] A. H. Corwin,[13] J. Kuck and E. Loewenstein,[23] and others [3, 4, 6, 8, 10, 12, 17, 18, 21, 46] should be consulted.

Aside from the Kuhlmann type of microanalytical balances,[24] for which the term *microchemical balance* has been in vogue, a number of differently constructed microanalytical balances serving a like purpose have been described and are in actual use.[15, 20, 23, 38, 48] One such balance [23] possesses a new rider device according to the principle of L. Ramberg.[38] It has a short beam with only forty notches on the rider scale. A 2-milligram quartz rod is used as rider.[35, 39]

F. Emich,[16] in his first quantitative inorganic microprocedures, used the microbalance of W. Nernst [31, 32] which had to be calibrated empirically. Quartz spring balances of the Salvioni type can be utilized for milligram procedures and have been found particularly useful for demonstrations in lectures. A practical quartz fiber microbalance has been described by J. Donau.[15] Precision balances of various designs have been used since 1886 [47] for quantitative analytical procedures involving milligrams and smaller amounts of material.[5, 20, 25, 44] For quantitative microgram procedures, such as residue determinations and electrolysis, F. Emich and E. Wiesenberger [48] employed a modified Nernst microbalance.

LITERATURE

1. Ainsworth, A. W., *Ind. Eng. Chem., Anal. Ed.*, **11**, 572 (1929); Laboratory, **11**, 54 (1940).
2. Ainsworth, Wm., and Sons, Inc., Denver, Colorado, U. S. A.
3. Alber, H. K., Mimeographed Outlines, Dr. J. B. Niederl's Microchemical Laboratory, New York University, 1936.

4. Alber, H. K., and Harand, J., *Ind. Eng. Chem., Anal. Ed.*, **10**, 405 (1938); *J. Franklin Inst.*, **224**, 729 (1937).
5. Ångström, A. T., *Svensk Vetensk. Selsk. Forhandling*, **9**, 643 (1895).
6. Anonymous, *Tech. Blätter, Bergwerks Ztg.*, **27**, 742 (1937).
7. Becker, C., Inc., Jersey City, N. J., U. S. A.
8. Benedetti-Pichler, A. A., "Die Fortschritte der Mikrochemie in den Jahren 1915–1926," F. Deuticke, Vienna, 1927, pp. 158–164; *Mikrochemie*, **25**, 390 (1938); *Ind. Eng. Chem., Anal. Ed.*, **11**, 226 (1939).
9. Benedetti-Pichler, A. A., and Paulson, R. A., *Mikrochemie*, **27**, 339 (1939).
10. Breuer, F., *Ind. Eng. Chem., Anal. Ed.*, **9**, 354 (1937).
11. Bunge, P., Hamburg, Germany.
12. Clarke, B. L., and Hermance, H. W., *Ind. Eng. Chem., Anal. Ed.*, **7**, 220 (1935).
13. Corwin, A. H., Chapel Hill Meeting, Am. Chem. Soc., April, 1937; Cincinnati Meeting, Am. Chem. Soc., April, 1940; *Mikrochemie*, **22**, 263 (1937); Metropol. Microchem. Soc., Lecture, May 23, 1940.
14. Craig, A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 581 (1939).
15. Donau, J., *Mikrochemie*, **9**, 1 (1931); **13**, 155 (1933).
16. Emich, F., "Lehrbuch der Mikrochemie," J. G. Bergmann, Munich, 1926, pp. 71–80; *Ber.*, **43**, 10 (1910).
17. Fuchs, L., *Mikrochemie*, **10**, 456 (1932).
18. Furter, M., *Mikrochemie*, **18**, 1 (1935).
19. Gorbach, G., *Mikrochemie*, **20**, 254 (1936); Monograph, "Die Mikrowage."
20. Johns, I. B., Rochester Meeting, Am. Chem. Soc., Sept. 9, 1937.
21. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **9**, 300 (1937); **5**, 363 (1933)
22. Kreider, L. C., *Ind. Eng. Chem., Anal. Ed.*, **13**, 117 (1941).
23. Kuck, J., and Loewenstein, E., *J. Chem. Education*, **17**, 171 (1940).
24. Kuhlmann, Wm. H. F., Steilshoper Str. 101–103, Hamburg, Germany
25. Lenz, W., *Apoth. Ztg.*, **21**, 23 (1912).
26. Longue, C. (successeur de A. Collot), Paris, France.
27. Moriya, K., Tokyo, Japan.
28. Moyer, H. V., *Ind. Eng. Chem.*, **17**, 540 (1940).
29. Müller, R. H., *Ind. Eng. Chem.*, **12**, 620 (1940).
30. Nemetz, J., Vienna, Austria.
31. Nernst, W., *Göttinger Nachrichten*, 1903, pp. 75–82.
32. Nernst, W., and Riesenfeld, E. H., *Ber.*, **36**, 2086 (1903).
33. Niederl, J. B., Niederl, V., Nagel, R. H., and Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).
34. Oertling, L., Ltd., 65 Holborn Viaduct, London, England.
35. Pfaltz and Bauer, Inc., 350 Fifth Avenue, New York, N. Y., U. S. A.
36. Power, F. W., *Mikrochemie*, **25**, 389 (1938); Milwaukee Meeting, Am. Chem. Soc., September, 1938.
37. Pregl, F., *Ber.*, **44**, 553 (1911); "Die quantitative organische Mikroanalyse," J. Springer, Berlin, 1930, Third Edition, pp. 7–19; (*a*) "Abderhalden's Handbuch der biologischen Arbeitsmethoden," Urban and Schwarzenberg, Vienna and Berlin, 1912, Vol. 5, p. 1307.
38. Ramberg, L., *Svensk Kem. Tidk.*, **41**, 106 (1929); **44**, 188 (1932); *Arkiv Kemi, Mineral. Geol.* **11-*A***, 7 (1933).
39. Sartorius Werke A. G., Göttingen, Germany.
40. Schulze, P., *Z. Instrumentenk.*, **12**, 97 (1892).
41. Schwarz-Bergkampf, E., *Z. anorg. Chem.*, **143**, 247 (1925).

42. Starke and Kammerer, A., G., Karlsgasse 11, Vienna, IV, Austria.
43. Seederer-Kohlbusch, Jersey City, N. J., U. S. A.
44. Steele, B. D., and Grant, K., *Proc. Roy. Soc. (London)*, **82**, 580 (1909).
45. Sternberg, H., *Mikrochemie*, **22**, 187 (1937).
46. van Straten, F. W., and Ehret, W. F., *Ind. Eng. Chem., Anal. Ed.*, **9**, 443 (1937).
47. Warburg, E., and Ihmori, T., *Ann. Phys. Chem.*, **27**, 481 (1886).
48. Wiesenberger, E., *Mikrochemie*, **10**, 10 (1932).
49. Wise, L. E., *J. Am. Chem. Soc.*, **39**, 2055 (1917).

WEIGHING

MICROANALYTICAL BALANCE

The microanalytical balance is considered to be in equilibrium when the 5-mg. rider rests in the first notch, which is marked zero, at the left end of the beam. Moving the rider for one notch to the right (0.1 mg.) causes a deflection of ten *divisions* of the pointer on the pointer scale, provided that the sensitivity of the balance is exactly 100. One *division* on the pointer scale thus represents 0.01 mg. Each *division* on the pointer scale is counted as 10 *deflection units*. A single *deflection unit* or "pointer-scale unit," [1a] is thus regarded to equal 0.001 mg., or 1 microgram, or 1 gamma (γ).

The pointer scale is calibrated to be read in the following manner: The center line where the pointer stops when the balance is arrested is considered zero; each division to the right of this zero line is counted as +10 and to the left as −10 deflection units. In practice, as a matter of simplicity, it is expedient to interpret the readings on the pointer scale as whole numbers and not as fractions, as for example, a swing of the pointer for 5½ divisions is read as 55 deflection units, that is 55 micrograms.

The arresting mechanism of the balance functions in such a way that, when the handle attached to the arrestment mechanism outside of the balance case is partly turned, only the pans are released. If they start swinging, indicating that they are off center, they are arrested again, and this operation of arresting and releasing is repeated until the pans remain stationary. Then the balance is released completely by turning the handle backward until it comes to a stop. By releasing the arrestment properly, the amplitude of the swings of the pointer, which normally should be between 3 and 8 divisions, can be controlled; at no time should the pointer be permitted to swing beyond the scale. If the amplitude of the swings exceeds 5 divisions on any one side of the zero line, thereby giving a deflection sum in excess of 50, then the rider should be placed on the adjacent notch to obtain a deflection sum of less than 50. Upon release of the arrestment of the balance the first two swings of the pointer are ignored when the pointer started to swing from the left, and three, when from the right. The next swing, beginning from the left, is counted, and the point of reversal of the pointer noted as illustrated below:

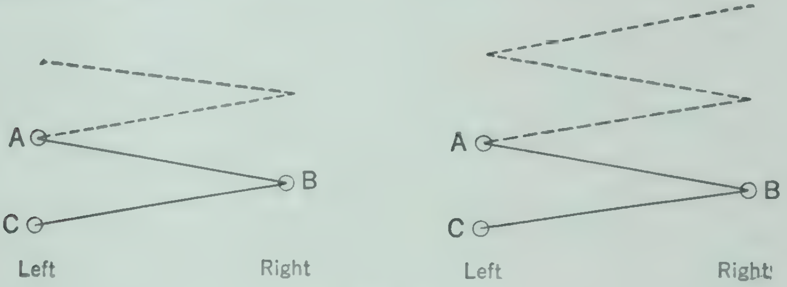


Fig. 2.

Position *A*: point of reversal at the left.
Position *B*: point of reversal at the right.
Position *C*: point of reversal at the left.

The *deflection sum* * is the algebraic sum of position *B* and the average of positions *A* and *C*.

After the readings have been taken the beam is arrested gradually when the pointer swings toward the zero line of the pointer scale. The deflection sum is *added* to the beam reading if *positive*, and *deducted* from the beam reading if *negative*, as illustrated by the following four examples:

Case	Point of Reversal *to the left* (*of the zero line*)	Point of Reversal *to the right* (*of the zero line*)	Position *A*	Position *C*	Average of *AC*	Position *B*	Deflection Sum	
1		All	+ 3	+ 5	+ 4	+23	+27	to be added
2	All		−42	−40	−41	− 7	−48	to be subtracted
3	*A,C*	*B*	−15	−13	−14	+30	+16	to be added
4	*A*	*B,C*	− 1	+ 3	+ 1	+30	+31	to be added

With the rider on the rider scale at 5.4 (beam reading: 5.4 mg.) the final results of the above four weighings would be as follows: case 1: 5.427 mg.; case 2: 5.352 mg.; case 3: 5.416 mg.; case 4: 5.431 mg.

Determination of the Zero Reading

The *zero reading* is defined as the *deflection sum* obtained when the balance is released with the rider at zero and the balance pans empty. The zero reading differs from the so-called zero point, which is actually one-half of the zero reading.

---

* The expression *deflection sum* used in this book is synonymous with the expressions *deflection*, *deflection difference*, *mean deflection*, or *Ausschlag*, used by other authors.

After the side doors of the balance have been closed the rider is placed on the first notch (0). The rider should sit straight and rest on the lowest point of the notch; to insure this the rider may have to be lifted up again and be permitted to fall into the notch, or it may be touched lightly at its apex with the hook of the rider carrier. The arresting mechanism is then released and the swings of the pointer on the pointer scale observed. This procedure is repeated until no greater deviations than ±2 *deflection units* are obtained in three successive trials. This repetition of the weighings, or *checking*, is a general rule which should always be observed. If more than 30 minutes pass between two weighings of the same object, it is necessary to redetermine the zero reading and to correct for any possible deviation as explained below:

ZERO READING			
First	*Second*	CHANGE	
−30	−25	5	to be subtracted
−22	−31	9	to be added
+17	+28	11	to be subtracted
+35	+27	8	to be added

Determination of the Sensitivity and Precision

The sensitivity * of the balance is equal to the *algebraic difference* of the two deflection sums with the rider at the zero and 0.1 mg. notch, respectively.

EXAMPLE:

Deflection sum (at notch 0)	+12
Deflection sum (at notch 0.1 mg.)	−(−84)
Sensitivity of the balance	96

If the zero reading is negative, a small object such as a lead shot is placed on the left balance pan and weighed with the rider at two adjacent notches so that both plus and minus readings are obtained. As shown in the following example:

FIRST DETERMINATION		SECOND DETERMINATION	
Beam reading	5.300 mg.	Beam reading	5.400 mg.
Deflection sum	+0.021 mg.	Deflection sum	−0.075 mg.
Weight	5.321 mg.	Weight	5.325 mg.

The sensitivity of the balance in the above case is also 96 [21 − (−75)]. The sensitivity with a load of 10 grams is determined in a similar manner.

---

* The term *sensitivity* is to be interpreted as being synonymous with *sensibility*, a term also used in connection with analytical balances.[8]

The precision of the microanalytical balance[1, 2, 9, 24] is determined by taking a series of at least twelve readings, but always alternating the rider position between 0.0 mg. and 0.1 mg., so that six readings of each rider position are obtained.

The following tables illustrate a typical application of the foregoing procedure of determining the sensitivity and precision of a microanalytical balance.

DETERMINATION OF THE SENSITIVITY AND PRECISION

No Load

Rider at 0.0	*Deflection Sum*	*Rider at* 0.1	*Deflection Sum*
1. +4 + 27 +5 +5	+ 5 + 27 = + 32	2. −53 − 11 −49 −51	− 51 + (−11) = − 62
3. +2 + 26 +4 +3	+ 3 + 26 = + 29	4. −57 − 13 −52 −55	− 55 + (−13) = − 68
5. +2 + 32 +4 +3	+ 3 + 32 = + 35	6. −50 − 12 −48 −49	− 49 + (−12) = − 61
7. +2 + 32 +6 +4	+ 4 + 32 = + 36	8. −42 − 15 −39 −41	− 41 + (−15) = − 56
9. +3 + 31 +5 +4	+ 4 + 31 = + 35	10. −51 − 12 −49 −50	− 50 + (−12) = − 62
11. +2 + 30 +6 +4	+ 4 + 30 = + 34	12. −51 − 12 −48 −50	− 50 + (−12) = − 62

SENSITIVITY

Weighings

No. 1 and 2: 32 − (−62) = 94
3 and 4: 29 − (−68) = 97
5 and 6: 35 − (−61) = 96
7 and 8: 36 − (−56) = 92
9 and 10: 35 − (−62) = 97
11 and 12: 34 − (−62) = 96

Average: 96

Sensitivity

0.1 milligram = 96 deflection units
1 deflection unit = 1.04 microgram (1/96)

PRECISION

Weighings		*Deviation from Average*	*Weighings*		*Deviation from Average*
No. 1:	+32	1	No. 2:	−62	0
3:	+29	4	4:	−68	6
5:	+35	2	6:	−61	1
7:	+36	3	8:	−56	6
9:	+35	2	10:	−62	0
11:	+34	1	12:	−62	1
Average:	33	13 ÷ 6 = 2.2	Average:	62	13 ÷ 6 = 2.2

*Precision:**

No better than ±2 deflection units (±2.08 micrograms)

10-GRAM LOAD

Rider at 0.0	*Deflection Sum*	*Rider at* 0.1	*Deflection Sum*
1. 0 + 67 +6 +3	+ 3 + 67 = + 70	2. −32 + 15 −28 −30	− 30 + 15 = − 15
3. +5 + 60 +9 +7	+ 7 + 60 = + 67	4. −30 + 14 −25 −27	− 27 + 14 = − 13
5. +1 + 68 +7 +4	+ 4 + 68 = + 72	6. −41 + 31 −35 −38	− 38 + 31 = − 7
7. +3 + 60 +9 +6	+ 6 + 60 = + 66	8. −29 + 14 −24 −26	− 26 + 14 = − 12
9. 0 + 64 +6 +3	+ 3 + 64 = + 67	10. −32 + 15 −26 −29	− 29 + 15 = − 14
11. +2 + 61 +7 +4	+ 4 + 61 = + 65	12. −39 + 26 −33 −36	− 36 + 26 = − 10

---

* The precision (reproducibility of weighings) of a balance cannot be better than its sensitivity and should be expressed in whole deflection units, since it is not possible to read the pointer scale more accurately than ±1 deflection unit. To judge the weighing performance of a microanalytical balance or an ordinary analytical balance used in micro work, *both* the sensitivity and precision must be given.

Sensitivity

Weighings

No. 1 and 2: 70 − (−15) = 85
3 and 4: 67 − (−13) = 80
5 and 6: 72 − (− 7) = 79
7 and 8: 66 − (−12) = 78
9 and 10: 67 − (−14) = 81
11 and 12: 65 − (−10) = 75
Average: 80

Sensitivity

0.1 milligram = 80 deflection units
1 deflection unit = 1.25 microgram (1/80)

Precision

Weighings		*Deviation from Average*
No. 1:	+70	2
3:	+67	1
5:	+72	4
7:	+66	2
9:	+67	1
11:	+65	3
Average:	68	13 ÷ 6 = 2.2

Weighings		*Deviation from Average*
No. 2:	−15	3
4:	−13	1
6:	− 7	5
8:	−12	0
10:	−14	2
12:	−10	2
Average:	12	13 ÷ 6 = 2.2

Precision:

Not better than ± 2 deflection units (± 2.5 micrograms)

The foregoing experiments indicate that this particular microanalytical balance has a sensitivity of 96 when without a load, meaning that 1 deflection unit corresponds to 1/960 mg., or 1.04 microgram. This would indicate good balance performance; however, the precision experiments indicate that weighing results cannot be reproduced better than within ±2 deflection units (±2.08 micrograms) at no load. With a 10-gram load the sensitivity dropped to 80, with 1 deflection unit corresponding to 1.25 microgram, while the precision, reproducibility of weighings, still remained at ±2 deflection units which now represent ±2.5 micrograms. In other words a sample can be weighed on this balance to within ±2.08 micrograms (maximum error: 4.16 micrograms) and an object weighing not more than 10 grams to within ±2.5 micrograms (maximum error: 5.0 micrograms).

The foregoing sensitivity and precision determinations are actual students' (beginners') experiments, performed on a microanalytical balance which has been in continuous use for sixteen years.

Sensitivity Correction Table

The sensitivity of a microanalytical balance is seldom precisely 100, but more often between 80 and 105. If the sensitivity is between 95 and 105, it is not necessary to correct for it because then the error is within the error of estimation of the deflection units. However, if the sensitivity is either below or above these figures, then the necessary corrections should be applied. For instance, if the sensitivity of the balance is 82, the correction is obtained by dividing the theoretical sensitivity (100) by the actual sensitivity (82), $100 \div 82 = 1.22$; therefore, each 0.001 mg. or, better, each deflection unit, represents an actual value of 0.00122 mg., and 0.00022 mg. is added to every 0.001 mg. irrespective whether the reading is plus or minus. In actual practice however, a correction is made only if the error due to the deviation of the actual sensitivity from the theoretical 100 exceeds the precision required for the object to be weighed, and then the necessary correction should be applied as shown in the correction table below. Such a correction table needs to be calculated only to 50, the practical deflection sum limit.

Correction Table

Actual Reading (Deflection Units)	*Corrected Values (Micrograms)*
1	1
5	6
10	12
15	18
20	24
30	37
40	49
50	61

Adjustment of the Sensitivity

If the sensitivity of the microanalytical balance with no load falls below 80 it may be necessary to adjust its sensitivity to, or near to, the theoretical 100. It is a rather difficult and tedious assignment to adjust the sensitivity to exactly 100, but since a balance having a sensitivity ranging between 95 and 105 permits weighing without the necessity of making sensitivity corrections, an adjustment between the above figures is entirely satisfactory.

To adjust the sensitivity of the microanalytical balance, first the pans, then the stirrups and the beam, are removed. The beam is held at the terminals between the index finger and thumb of the left hand and, with the rider scale facing the operator, the sensitivity screw back of the beam is turned to the left, or upward, if the sensitivity is to be raised, and to the right, or downward, if it is to be lowered. A lowering of the sensitivity is seldom necessary. No definite statement can be made just how much the screw should be turned to obtain a given sensitivity, but as a rule, a quarter turn in the proper direction will raise or lower the sensitivity by about 5. It is advisable to write down not only how much the screw has been turned, but also the direction in which it was turned, because this operation, including the testing which follows, may take several days before a proper adjustment is obtained and one may forget in the ensuing intervals just what has been done. Furthermore, the sensitivity screw should never be given more than a quarter turn at one time, because only then will the operator become aware of the respective changes of the sensitivity obtained, a fact which not only will be of value when future adjustments are attempted, but also will prevent a destructive disturbance of the sensitivity mechanism. It should also be emphasized that the sensitivity screw should be turned with the fingers and not with pliers or forceps and that all application of force must be avoided. The balance is then reassembled and the change in the sensitivity determined after an elapse of at least three hours. If necessary the above operation is repeated until the desired sensitivity has been obtained.

Should the balance show the proper sensitivity at no load but drop considerably with a 10-gram load—that is, drop more than 10 points—then an adjustment as described above will be of no avail, because under such conditions the balance, that is, the beam as well as the knife edges, may show defects which can be repaired only by balance experts or by the manufacturer of the balance.

ORDINARY ANALYTICAL BALANCE

An ordinary analytical balance suitable for quantitative micro determinations may be selected from a number of balances available, by systematically determining their sensitivity and precision in the manner outlined below and then choosing one which conforms to, or still better exceeds, the practical minimum requirements of precision stated on p. 6. Also any of the so-called *semi-micro* or *precision analytical* balances available on the market may be used, but nevertheless should be subjected to the same determination of precision and sensitivity.

Since the weighings on such an analytical balance are performed exactly like those on the microanalytical balance, its beam should have the customary 10-mg. rider scale, the zero mark being at the left terminal, the 5-mg. mark in the center, and the 10-mg. mark at the right terminal. Only the whole milligram notches are necessary, and hence the subdivisions denoting tenths of a milligram may be omitted. Conforming to the above arrangement of the rider scale, the balance must be adjusted so that it is in equilibrium when the rider rests on the zero mark at the left end of the beam. The pointer scale of such a balance should possess accurately spaced divisions, because it is read just like the pointer scale of the microanalytical balance. The value for each *deflection unit*, one-tenth of one division on the pointer scale, in terms of micrograms has to be determined separately for every balance. Therefore, depending upon the sensitivity and precision of such a balance, a single *deflection unit*—the estimated tenth of each division—may correspond to anywhere between 5 and 20 micrograms.

Determination of the Zero Reading

As with the microanalytical balance, the zero reading of an ordinary analytical balance to be used in micro work is also defined as the *deflection sum* determined with the balance pans empty and the 5-mg. rider at the zero mark. A zero reading taken in this manner differs from the actual zero point, which corresponds to half of the zero reading. The procedure for this determination, that is the reading of the point of reversal of the pointer, is exactly the same as given for the determination of the zero reading of the microanalytical balance. The *deflection units* of every swing of the pointer on the pointer scale are noted and from the figures obtained the *deflection sum* is calculated. In this case the deflection sum denotes merely deflection units, the value of which in terms of milligrams, or micrograms, is calculated after the sensitivity and precision of the balance have been determined.

Determination of the Sensitivity and Precision

The sensitivity and precision of an analytical balance intended for use in quantitative microanalysis are determined in the same manner as described for the microanalytical balance. The rider, however, is shifted one whole milligram division, instead of 0.1 mg. as with the microanalytical balance.

The tables below furnish an actual example of the results obtained from an analytical balance used in microanalytical work.

DETERMINATION OF SENSITIVITY AND PRECISION

No Load

Rider at 0.0	*Deflection Sum*	*Rider at* 1.0	*Deflection Sum*
1. $\begin{array}{r}-32\\-29\\\hline -31\end{array} + 46$	$+46 - 31 = +15$	2. $\begin{array}{r}-96\\-93\\\hline -95\end{array} + 6$	$-95 + 6 = -89$
3. $\begin{array}{r}-16\\-14\\\hline -15\end{array} + 31$	$+31 - 15 = +16$	4. $\begin{array}{r}-114\\-112\\\hline -113\end{array} + 26$	$-113 + 26 = -87$
5. $\begin{array}{r}-28\\-26\\\hline -27\end{array} + 42$	$+42 - 27 = +15$	6. $\begin{array}{r}-97\\-94\\\hline -96\end{array} + 7$	$-96 + 7 = -89$
7. $\begin{array}{r}-24\\-23\\\hline -24\end{array} + 39$	$+39 - 24 = +15$	8. $\begin{array}{r}-103\\-100\\\hline -102\end{array} + 12$	$-102 + 12 = -90$
9. $\begin{array}{r}-19\\-17\\\hline -18\end{array} + 33$	$+33 - 18 = +15$	10. $\begin{array}{r}-94\\-92\\\hline -93\end{array} + 4$	$-93 + 4 = -89$
11. $\begin{array}{r}-61\\-57\\\hline -59\end{array} + 73$	$+73 - 59 = +14$	12. $\begin{array}{r}-94\\-93\\\hline -94\end{array} + 5$	$-94 + 5 = -89$

SENSITIVITY

Weighings

No. 1 and 2: $15 - (-89) = 104$
3 and 4: $16 - (-87) = 103$
5 and 6: $15 - (-89) = 104$
7 and 8: $15 - (-90) = 105$
9 and 10: $15 - (-89) = 104$
11 and 12: $14 - (-89) = 103$

Average: 104

Sensitivity

1 milligram = 104 deflection units
1 deflection unit = 9.6 micrograms (1/104)

PRECISION

Weighings		*Deviation from Average*	*Weighings*		*Deviation from Average*
No. 1:	+15	0	No. 2:	−89	0
3:	+16	1	4:	−87	2
5:	+15	0	6:	−89	0
7:	+15	0	8:	−90	1
9:	+15	0	10:	−89	0
11:	+14	1	12:	−89	0
Average:	+15	2 ÷ 6 = 0.33	Average	−89	3 ÷ 6 = 0.5

Precision:

Not better than ±1 deflection unit (±9.6 micrograms)

10-GRAM LOAD

Rider at 0.0	*Deflection Sum*	*Rider at* 1.0	*Deflection Sum*
1. $\begin{array}{r} -17 \\ -16 \\ \hline -16 \end{array} + 19$	$+19 - 16 = +3$	2. $\begin{array}{r} -98 \\ -95 \\ \hline -96 \end{array} - 5$	$-96 + (-5) = -101$
3. $\begin{array}{r} -18 \\ -16 \\ \hline -17 \end{array} + 19$	$+19 - 17 = +2$	4. $\begin{array}{r} -104 \\ -100 \\ \hline -102 \end{array} \quad 0$	$-102 + (\ \ 0) = -102$
5. $\begin{array}{r} -15 \\ -15 \\ \hline -15 \end{array} + 18$	$+18 - 15 = +3$	6. $\begin{array}{r} -95 \\ -93 \\ \hline -94 \end{array} - 6$	$-94 + (-6) = -100$
7. $\begin{array}{r} -21 \\ -19 \\ \hline -20 \end{array} + 24$	$+24 - 20 = +4$	8. $\begin{array}{r} -100 \\ -97 \\ \hline -99 \end{array} - 2$	$-99 + (-2) = -101$
9. $\begin{array}{r} -18 \\ -16 \\ \hline -17 \end{array} + 20$	$+20 - 17 = +3$	10. $\begin{array}{r} -97 \\ -94 \\ \hline -96 \end{array} - 3$	$-96 + (-3) = -99$
11. $\begin{array}{r} -25 \\ -23 \\ \hline -24 \end{array} + 26$	$+26 - 24 = +2$	12. $\begin{array}{r} -101 \\ -98 \\ \hline -99 \end{array} - 2$	$-99 + (-2) = -101$

Sensitivity

Weighings

No. 1 and 2: 3 − (−101) = 104
3 and 4: 2 − (−102) = 104
5 and 6: 3 − (−100) = 103
7 and 8: 4 − (−101) = 105
9 and 10: 3 − (− 99) = 102
11 and 12: 2 − (−101) = 103

Average: 103

Sensitivity

1 milligram = 103 deflection units
1 deflection unit = 9.7 micrograms (1/103)

Precision

Weighings		*Deviation from Average*	*Weighings*		*Deviation from Average*
No. 1:	+3	0	No. 2:	−101	0
3:	+2	1	4:	−102	1
5:	+3	0	6:	−100	1
7:	+4	1	8:	−101	0
9:	+3	0	10:	− 99	2
11:	+2	1	12:	−101	0
Average:	3	3 ÷ 6 = 0.5	Average:	101	4 ÷ 6 = 0.66

Precision:

Not better than ±1 deflection unit (±9.7 micrograms)

The foregoing weighing experiments indicate that this particular analytical balance, which has been in continuous use for three years, has a sensitivity of 104 deflection units, or 1/104 mg., or 9.6 micrograms, with no load, and that the weighings of a sample on this balance can be reproduced to within ±1 deflection unit, or ±9.6 micrograms. Both sensitivity and precision of this balance remained practically unchanged at a 10-gram load (±1 deflection unit; ±9.7 micrograms).

ACTUAL WEIGHING

To permit equalization of possible temperature differences between the inside of the balance case and the balance room, the side doors of the balance are half opened about 10 minutes before weighing. For the same reason they should be opened again as soon as the actual weighing has been completed.

The object to be weighed is placed on the left-hand weighing pan (boat) or on the hooks of this pan (tubes). Whenever possible the object should be introduced through the side doors, both of which should always be opened when this is done; objects which are placed on the hooks have to be introduced through the front. The counterpoise is then placed on the right-hand weighing pan and the doors of the balance are closed. After the rider has been placed on the 5-mg. mark the arresting mechanism is slowly released and the movement of the pointer observed. If the pointer swings to the right and beyond the scale, the balance is arrested and the rider moved to the 7.5-mg. notch. If, upon release of the arrestment, the pointer still swings too far to the right the rider is placed on the 10-mg. mark and the observation repeated; should the pointer still swing beyond the scale, then either a weight, such as the 10-mg. piece, is placed on the right-hand pan or the object is recounterpoised.

When, with the rider on the 5-mg. mark, the pointer swings to the left and off the pointer scale, then the rider is moved to 2.5 mg. and the observation repeated. If the pointer swings too far to the right, the rider is moved to a notch corresponding to half of the last two rider positions, in this case to 3.7 mg., and this division is continued until two limiting rider positions are found, one, the lower, when the pointer swings to the right, but no longer off the scale, and one, the higher, when the pointer, also remaining within the range of the scale divisions, swings to the left of the zero line of the pointer scale. Finally, a rider position which gives the smaller *deflection sum*, that is 50 or less, is chosen. On the ordinary analytical balance the rider is moved whole milligram divisions until the correct rider position is found.

After the *correct* position of the rider on the rider scale has been determined the average *deflection sum* is determined from three succeeding deflection sums, the balance being arrested after each determination of the deflection sum. The three *deflection sums* thus taken consecutively should agree within ±2 or ±3 deflection units on the microanalytical balance and within ±1 or at the most ±2 deflection units on the analytical balance.

The *deflection sum* is added to the value of the beam reading if positive, or plus, and deducted from the beam reading if negative, or minus. Practical examples of such weighings performed on both balances are given below.

MICROANALYTICAL BALANCE

Series of Pointer Swings

	First		*Second*		*Third*	
	−16	+ 59	−7	+ 51	−20	+ 61
	−10		−1		−13	
Average:	−13		−4		−16	

Deflection Sum

$$\begin{array}{rrr} +59 & +51 & +61 \\ -13 & -\ 4 & -16 \\ \hline +46 & +47 & +45\ (\text{Average} = +46) \end{array}$$

Actual Weight:

Position of the rider:	1.500 mg.
Deflection sum:	0.046
Weight:	1.546 mg. (1 deflection unit = 0.001 mg.)

ORDINARY ANALYTICAL BALANCE

Series of Pointer Swings

$$\begin{array}{rccc} & \textit{First} & \textit{Second} & \textit{Third} \\ & \begin{array}{r}-31\\-29\end{array} + 38 & \begin{array}{r}-14\\-12\end{array} + 19 & \begin{array}{r}-21\\-20\end{array} + 29 \\ \cline{2-4} \text{Average:} & -30 & -13 & -21 \end{array}$$

Deflection Sum

$$\begin{array}{rrr} +38 & +19 & +29 \\ -30 & -13 & -21 \\ \hline +\ 8 & +\ 6 & +\ 8\ (\text{Average} = +7) \end{array}$$

Actual Weight:

Position of the rider:	1.00 mg.	
Deflection sum 7 × 0.0096 mg.	0.07	(1 deflection unit = 0.0096 mg.)
Weight:	1.07 mg.	

Calibration of Weights

It is necessary to check the weight of the 10-mg., the two 20-mg., and the 50-mg. pieces against the rider, because they seldom are in absolute agreement with the weight they represent. Since they are placed on the right pan when used in an actual weighing, the weights must be calibrated under identical conditions. This is accomplished by employing the substitution method of T. W. Richards,[16, 22, 26] which eliminates complicated calculations found in other procedures.[8, 9, 15, 16, 18, 28, 29] Two sets of weights are necessary: set W, the weights to be calibrated, consisting of one 10-mg. (W_{10}), two 20-mg. (W_{20} and $W_{20'}$), and one 50-mg. piece (W_{50}); set T, the weights used as the tare, comprising one 10-mg. (T_{10}), one 20-mg. (T_{20}), and one 50-mg. piece (T_{50}).

The 10-mg. weight of set T (T_{10}) is placed on the left-hand pan, while the rider is at the 10-mg. notch; readings are taken and the deflection sum (d_1) noted. Then the 10-mg. weight of set W (W_{10}), the weight to be calibrated, is placed on the right-hand pan and the rider moved to the zero notch; readings are again taken and the deflection sum (d_2) noted. The 10-mg. weight (T_{10}), which was used as the tare, is then replaced by the 20-mg. weight of set T (T_{20}); the calibrated 10-mg. weight (W_{10}) is placed on the right-hand pan and the rider inserted in the 10-mg. notch; readings are again taken. The 10-mg. weight is replaced by the 20-mg. weight of set W (W_{20}), the rider moved to zero, and the deflection sum noted. This procedure is repeated with the second 20-mg. weight of set W ($W_{20'}$). In calculating the results the corrected weight of the 10-mg. weight (W_{10}) has to be applied.

The calibration of the 50-mg. weight of set W (W_{50}) is carried out in the same manner, by placing the 50-mg. weight of set T (T_{50}) on the left-hand pan and the two 20-mg. weights of set W (W_{20} and $W_{20'}$) on the right-hand pan, while the rider is at 10 mg. The two weights on the right-hand pan are then replaced by the 50-mg. weight of set W (W_{50}) and the rider moved to the zero notch; again the corrected weight of the two 20-mg. weights has to be applied in the calculation. When the procedure of weighing has been completed, the results are tabulated as shown below and the correct weight of the pieces of set W calculated according to the following equation:

$$T = C + \frac{d_1}{1000} \qquad T = W + \frac{d_2}{1000}$$

$$C + \frac{d_1}{1000} = W + \frac{d_2}{1000} \qquad W = C + \frac{d_1 - d_2}{1000}$$

T = tare.
W = weight to be calibrated.
C = rider, or rider plus weight on right-hand pan when calibrating the larger weights.
d_1 = deflection sum with rider at 10.
d_2 = deflection sum with rider at 0 and weight to be calibrated on right-hand pan.

	Tare, mg.	*Weight, mg.*	*Rider*	*Deflection Sum*	*Corrected Weight*
I.	10.000	No weight	10	−31	
	10.000	10.000	0	−36	W_{10} 10.005 mg.

$$W = 10.000 + \left(\frac{-31 + 36}{1000}\right) = 10.000 + \frac{5}{1000} = 10.005$$

	Tare, mg.	*Weight, mg.*	*Rider*	*Deflection Sum*	*Corrected Weight*
II.	20.000	10.005	10	−26	
	20.000	20.000	0	−14	W_{20} 19.993 mg.

$$W = 20.005 + \left(\frac{-26 + 14}{1000}\right) = 20.005 - \frac{12}{1000} = 19.993$$

	Tare, mg.	*Weight, mg.*	*Rider*	*Deflection Sum*	*Corrected Weight*
III.	20.000	10.005	10	+18	
	20.000	20.000′	0	+ 8	W_{20}' 20.015 mg

$$W = 20.005 + \left(\frac{+18 - 8}{1000}\right) = 20.005 + \frac{10}{1000} = 20.015$$

	Tare, mg.	*Weight, mg.*	*Rider*	*Deflection Sum*	*Corrected Weight*
IV.		19.993			
		20.015			
	50.000	40.008	10	+12	
	50.000	50.000	0	+20	W_{50} 50.000 mg.

$$W = 50.008 + \left(\frac{+12 - 20}{1000}\right) = 50.008 - \frac{8}{1000} = 50.000$$

The accuracy of the aforementioned weights should be tested occasionally, and if their weight has increased materially they ought to be cleaned by washing with water and alcohol and polishing with a fine cloth. The weights should be handled only with the ivory-tipped forceps and kept in the balance case in a small dish lined with chamois. The rider may also change its weight; in this event the rider is washed in dilute potassium cyanide solution, dried, and gently rubbed between chamois-covered finger tips. Most of the time an increase in weight is observed; a decrease in weight occurs very seldom unless the rider is subjected to improper handling such as touching it with metal forceps; then it is best to replace it with a new one. Should the rider become bent, its shape may be restored by pressing it between several sheets of smooth, hard paper.

Preparation of Counterpoises

Since in quantitative analysis it is necessary to know only the weight of the sample and the weight of the reaction products, the weighing vessels are suitably counterpoised. The type of counterpoise varies with the type of weighing vessel used.

Theoretically, in order to minimize the buoyant effect of the air, the counterpoise should be of the same density as the object—metal for metal, glass for glass, porcelain for porcelain, etc.—and best also of the same shape and volume. Under adverse conditions the influence of variations in temperature and atmospheric pressure upon counterpoises may amount to as much as 20 to 40 micrograms.[7–14, 25, 30] In practice, however, metal wire is used for light objects, and especially designed glass containers, so-called tare bottles, preferably provided with a ground-glass stopper and filled with lead shot or glass rods, are used for heavier objects.

The counterpoise for light objects is made of a paper clip or any corrosion-resistant wire. The first adjustment is made on an ordinary analytical balance by clipping or filing off portions of the wire until it is about 1 mg. *heavier* than the object; then it is placed on the microanalytical balance and portions filed off until the counterpoise is about 1 mg. lighter than the object. The procedure for counterpoising with tare bottles is similar. The object is counterpoised on an ordinary analytical balance by adding lead shot to the tare bottle until it is about 10 mg. *lighter* than the object. For the final adjustment two sizes of lead shot are used, medium and fine, a single piece weighing approximately 10 or 5 mg., respectively. After the tare and object have been placed on the microanalytical balance a single medium-size lead shot is put beside the tare on the right-hand pan and the reading taken; lead shot is then added piece by piece until the bottle is about 1 mg. lighter than the object. The single lead shot which has been placed beside the tare bottle is then placed in the tare bottle. The properly marked counterpoises are kept in the balance case.

Numerous weighing procedures [4, 6, 11, 19, 27] have been described in the literature,[3, 7, 17, 18, 21, 23, 24, 25] as well as precautions to be observed when weighing on supersensitive balances. F. Breuer [5] advised the use of crucible tongs for the placing of objects on the balance, as well as elongation of the rider rod.

LITERATURE

1. Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **8**, 373 (1936); **11**, 226 (1939). (*a*) Private communication.
2. Benedetti-Pichler, A. A., and Paulson, R. A., *Mikrochemie*, **27**, 339 (1939).
3. Blade, E., *Ind. Eng. Chem., Anal. Ed.*, **11**, 499 (1939).
4. Booth, H. S., and Damerell, R. V., "Qualitative Analysis," McGraw-Hill Book Co., New York, 1940, pp. 22–23.
5. Breuer, F., *Ind. Eng. Chem., Anal. Ed.*, **9**, 354 (1937).
6. Brinton, L. H., *J. Am. Chem. Soc.*, **41**, 1151 (1919).
7. Corwin, A. H., Chapel Hill Meeting, Am. Chem. Soc., April, 1937; *Mikrochemie*, **22**, 263 (1937); Cincinnati Meeting, Am. Chem. Soc., April, 1940.
8. Eaton, F. C., *J. Am. Chem. Soc.*, **54**, 3261 (1932).
9. Fales, H. A., "Inorganic Quantitative Analysis," Century Co., New York, 1925.
10. Flaschenträger, W., *Z. anal. Chem.*, **83**, 422 (1931).
11. Harris, G. W., *J. Chem. Education*, **18**, 81 (1941).
12. Hayman, D. F., *Ind. Eng. Chem., Anal. Ed.*, **4**, 256 (1932); Rochester Meeting, Am. Chem. Soc., Sept. 9, 1937.
13. Heller, K., and Meyer, K., *Z. anal. Chem.*, **71**, 117 (1927).
14. Hernler, F., *Mikrochemie, Pregl Festschrift*, 1929, p. 140; *Emich Festschrift*, 1930, p. 148.
15. Hopkins, A., Zinn, J., and Rogers, H., *J. Am. Chem. Soc.*, **42**, 2528 (1920).
16. Hurley, F. H., *Ind. Eng. Chem., Anal. Ed.*, **9**, 239 (1937).

17. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **9**, 300 (1937).
18. Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," Macmillan Co., New York, 1936.
19. Lin, I., *J. Chem. Education*, **16**, 340 (1939).
20. Niederl, J. B., Niederl, V., Nagel, R. H., and Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).
21. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, pp. 7–19.
22. Richards, T. W., *J. Am. Chem. Soc.*, **22**, 144 (1900).
23. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 1–8.
24. Schwarz-Bergkampf, E., *Z. anal. Chem.*, **69**, 321 (1926).
25. Sternberg, H., *Mikrochemie*, **22**, 187 (1937).
26. Thornton, W. M., *J. Chem. Education*, **14**, 270 (1937).
27. Turner, R. R., *Chemist-Analyst*, **16**, 21 (1916).
28. Weatherill, P. F., *J. Am. Chem. Soc.*, **52**, 1938 (1930).
29. Willard, H. H., and Furman, N., "Elementary Quantitative Analysis," Second Edition, D. Van Nostrand Co., New York, 1935, p. 47.
30. Williams, R. J., *Ind. Eng. Chem., Anal. Ed.*, **8**, 229 (1936).

WEIGHING EQUIPMENT

The weighing equipment listed below is the *minimum* necessary and may be augmented by any of the many mechanical contrivances and gadgets already reported in the literature which fulfill similar purposes and which lend themselves to almost endless variations in design.

Forceps. Two kinds of forceps are needed. Forceps for handling the platinum boat and platinum foil should be all metal, of stainless steel; platinum tips are preferable but not absolutely essential. The second type is required for the handling of the weights. For this purpose ivory-tipped forceps which are furnished with each set of weights are in vogue. For a similar purpose and for handling capillaries, weighing tubes, weighing bottles, tares, etc., chamois-tipped forceps prepared as follows are recommended:[3] A pair of metal forceps is heated in the flame of a Bunsen burner and some Krönig's glass cement is applied to the tips, which must be hot enough so that the glass cement remains molten while a small piece of chamois is attached to the inside. The superfluous chamois is cut off.

For the manipulation of larger objects ordinary crucible tongs with or without platinum tips are used. To lessen temperature changes in the balance case when introducing objects, weighing tools with long handles have been suggested and have found practical application for working under adverse conditions.[2] A special type of forceps having tapered grooves at the tips has been found convenient for the handling of the various kinds of capillaries employed in quantitative organic microanalysis.[1]

Spatula. A plain metal rod of stainless steel, aluminum, platinum, or nickel of about 16-cm. length and 3-mm. diameter, flattened out at one end, is very convenient. A biological dissecting knife having a blade of 4- to 5-mm. diameter may be employed for the same purpose.

Probing Wire. A steel wire of 0.7- to 0.8-mm. diameter with knurled ends, around which cotton is wound, is an excellent device for cleaning the capillary ends of the absorption tubes, weighing tubes, etc. A toothpick quite often fulfills the same purpose.

Camel's-Hair Brush. A small camel's-hair brush is very convenient for the removal of particles adhering to the outer surface of the weighing boat or weighing tube.

Desiccator. Of the several types of desiccators [4, 5, 6, 7] available the following (Fig. 3) appears to be the most practical: [8] A round aluminum block of 62-mm. diameter and 45 mm. in height, has on its upper surface a concentric ring 6 mm. in diameter and 5 mm. in height; a 50-ml. flat-bottom crystallizing dish of 56-mm. outer diameter and 38-mm. height fits into the outer periphery of this ring and serves as a cover. In its center the metal block has a flat-bottom conical cavity, 35 mm. wide and 33 mm. deep, which serves as a convenient receptacle for crucibles used for the ignition of precipitates. The rim of this cavity is 5 mm. below the concentric ring and supports a concave copper block, of 39-mm. diameter and 12-mm. thickness, which serves as a support and efficient cooling device.

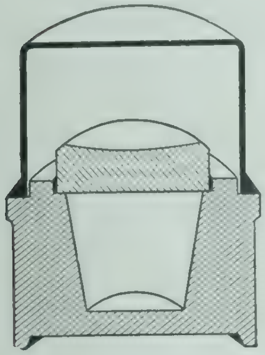


Fig. 3. Microdesiccator.

Platinum Wire. Platinum wire is bent at right angles at one end to form a small hook, the other end being fused into a glass rod of suitable length. The glass rod used for the introduction of platinum boats into the combustion tube is about 30 cm. long; the other for general use, such as cleaning of the platinum or porcelain boats, is about 20 cm. long, the wire for both being about 4 cm. long and 0.3 to 0.5 mm. in diameter.

Metal Tares. For counterpoising weighing boats and tubes, suitably bent rust-resistant wires are used. Ordinary paper clips have been found quite suitable for this purpose.

Tare Bottles. Tare bottles holding approximately 2 ml. for counterpoising absorption tubes, filter tubes, crucibles, etc., are approximately 18 to 20 mm. in diameter at the flat bottom and about 30 mm. in height with a neck 20 mm. in length and 6 mm. in diameter. Ordinary lead shot, as recommended by F. Pregl,[6] may be used. The tare bottles are made of soft glass and provided with a ground-glass stopper. Pyrex glass, on account of its property to retain electrical charges, cannot be used for this purpose. Although the buoyant effect is not taken into consideration in counterpoises of this type, they nevertheless serve for all ordinary purposes. If possible, however, the counterpoise should be of the same material as the object, and various contrivances fulfilling this particular requirement have been described.[9, 10, 11]

Rack. Since the absorption and filter tubes cannot be weighed immediately after their surface has been cleaned and wiped, a metal rack for their support should be included in the equipment. A penholder stand will serve this purpose very well. It is placed beside the balance on a piece of cardboard or notebook, because the temperature of the stone or metal table top usually differs from the temperature prevailing in the balance case, thereby adversely affecting the constancy of weight of the object. It is also advisable to ground the rack to permit the electrostatic charges which are produced by the wiping of the glass surface to dissipate.

Chamois, Flannel, Absorbent Cotton. Half a dozen pieces of chamois measuring 10 by 10 cm. should be available for cleaning the balance, wiping the absorption and filter tubes, handling the weighing tubes and tare bottles, etc. The chamois should be frequently washed with soap and rinsed with dilute ammonia water and distilled water. They are dried at room temperature by suspending them from a glass rod or a string and when dry are rubbed to softness. For protection from dust they are placed in covered glass jars or Petri dishes. Extraction of the chamois with ether or chloroform should not be resorted to, because they usually become inflexible by this treatment. Moist flannel cloth measuring 10 by 10 cm. is needed to clean the surface of the absorption and filter tubes; fiber-free linen cloth may be used for the same purpose. Two sets of chamois finger cots for thumbs and index fingers facilitate the handling of all weighing vessels as well as the cleaning of the balance and should, therefore, be included in the equipment.

Ordinary non-medicated absorbent cotton is suitable for the various purposes mentioned in the following chapters.

Brushes. A large brush for the cleaning of the balance and a good small camel's-hair brush for brushing the platinum boat and weighing tube, etc., are required.

LITERATURE

1. Alber, H. K., *Mikrochemie,* **18**, 92 (1935); *Ind. Eng. Chem., Anal. Ed.,* **10**, 348 (1938).
2. Breuer, F., *Ind. Eng. Chem., Anal. Ed.,* **9**, 354 (1937).
3. Cass, Wm. E., New York University, University Heights, New York, N. Y., private communication.
4. Corwin, A. H., Eimer & Amend Catalog, 1936, p. 488.
5. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, p. 41.
6. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, p. 19.
7. Röscheisen, P., and Breitner, P., *Mikrochemie,* **22**, 254 (1937); *Chem. Fabrik,* **10**, 321 (1937).
8. Saschek, W., *Ind. Eng. Chem., Anal. Ed.,* **9**, 491 (1937).
9. Sperry, W. M., *Mikrochemie,* **12**, 151 (1932).
10. Sternberg, H., *Mikrochemie,* **22**, 193 (1937).
11. Williams, R. J., *Ind. Eng. Chem., Anal. Ed.,* **8**, 229 (1936).

LABORATORY UTENSILS

Microburners. It is necessary to have several microburners available, not only for the preparation of the capillaries, micropipets, and small glass rods, but also for various heating and drying purposes. The burner recommended by F. Pregl [5, 12, 14, 17] is supported on four short legs and consists of a metal tube, one end of which is connected to a gas hose. Projecting at right angles from the side near the other end is a short gas jet with small air vents which take in sufficient air to give a nonluminous flame, which is reducible to a height of 3 mm. A screw controlling the flow of gas into the jet is inserted at this part of the metal tube. A vertical burner, incorporating the same principles, fulfills the purpose equally well, particularly if equipped with a needle valve. Several types of microburners are on the market, the most important feature of which is an exactly centered burner tip with sufficient but not too much air feeding. Microburners of Pyrex glass, of simple construction and easily made in the laboratory, work also well with natural gas [7] and are widely used. They are made by drawing out a glass tube of approximately 7-mm. inside diameter to a capillary of about 1-mm. inside diameter which may be further reduced to suit specific purposes. The capillary end is bent at right angles and the feeding glass tube mounted on a cork of suitable size. Bunsen burners with a pilot flame arrangement are also sufficient for many purposes. A Venable type burner may be adapted to micro work by unscrewing the barrel and using the small gas outlet in the base.[2]

Gas Burners. Aside from the long gas burner shown in Fig. 20, a Méker burner, for the preparation of capillaries from test tubes, and a blast burner are required. For the movable gas burner employed in the various combustion procedures and referred to in this manual as *gas burner*, any standard gas burner such as a *Tirrill, Pittsburgh Universal, Venable* type, *Fletcher*, or *Bunsen* burner will be adequate.

Drying Blocks. A large variety of drying blocks have been designed and are described in the literature.[2, 4, 10, 11, 12, 14] For the purpose of quantitative organic microanalysis the drying block of F. Pregl (Fig. 4),[12] which is used for the microvacuum desiccator and for drying of the filter tubes, has proved entirely satisfactory. It consists of two superimposed copper or aluminum blocks, each of which is provided with two parallel semicircular channels. One of these channels, having a diameter of approximately 13 mm., serves to hold the center portion of the microvacuum desiccator or the halogen filter tube. The second channel has a diameter of approximately 8 mm. and can be used for various purposes. The lower half of the block is mounted on a solid base approximately 9 cm. in height and is heated from below by means of a microburner. It also has a horizontal boring which serves as a receptacle for the thermometer. The upper half of the block is removable and is provided with a heat-insulated handle. Two wires, suitably bent, extend from the base and are intended as supports for objects protruding from the channels.

Hand Centrifuge. An ordinary hand centrifuge, such as employed in clinical work, is wholly adequate for operations involving the centrifuge cone, the filling of weighing capillaries, etc. The centrifuge cone is fitted into the larger metal tube by means of a suitably bored cork. The centrifuging of a capillary is best carried out by placing it in the centrifuge cone which has a protective cotton wad at the bottom to avoid breaking of the weighing capillary and which in turn is then inserted in the metal tube.

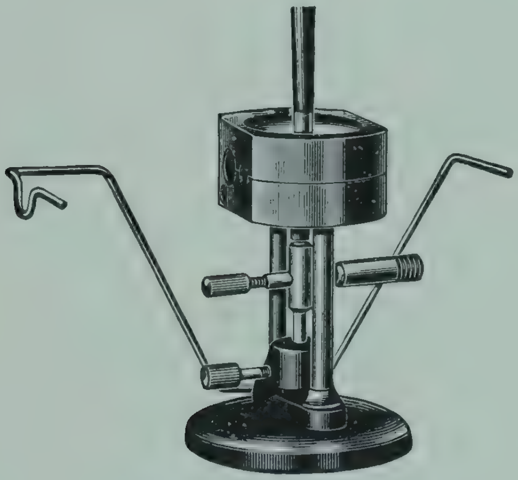


Fig. 4. Drying Block.

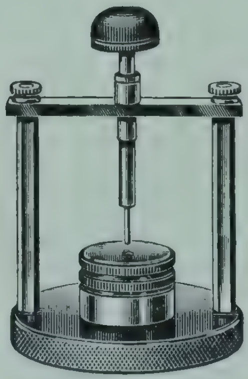


Fig. 5. Pellet Press

Pellet Press (Fig. 5). This apparatus [12] consists of a round, rustproof, horizontal metal base plate about 8.5 cm. in diameter, 12 mm. in height and 6 mm. thick. It is provided with a polished surface and a vertical support 5.3 cm. high. The horizontal cross bar of this support is provided with a vertical support to act as a guide for the plunger or compressor. The base plate has a circular impression 0.5 mm. deep and 3.6 cm. in diameter, as a support for the bottom metal block, which is 3.6 cm. in diameter and 1.5 cm. in height. This block has a cavity 2 cm. wide and 7 mm. deep in the center which serves as the receptacle for the finished pellet. The rim of this cavity is about 1 mm. lower than the edge of the base metal plate to support the cover block, which is 3.5 cm. wide and 1 cm. thick. A channel of 1-mm. bore is drilled through the center of the cover block through which passes the 12-mm.-long piston of the plunger. The substance to be compressed is introduced into this channel, and when filled it is pressed together by the plunger. During the process of the filling and pressing, the bottom block is placed upon the base in such a manner that its round cavity faces downward. A slight pressure is sufficient to form a coherent pellet 8 to 10 mg. in weight. In order to remove the pellet from the bore the position of the bottom block is reversed, so that in this instance the cavity faces the plunger. Re-insertion of the plunger into the bore of the upper block forces the pellet into the cavity of the bottom block. Then the pellet is cut into pieces weighing 3 to 5 mg., introduced into the weighing tube, weighed, and transferred to the reaction vessel. For reactive compounds, a pellet press made of glass [13] has been recommended.

Glass-Cutting Devices. For cutting glass tubings a triangle file usually suffices. Capillaries, however, are most conveniently cut with the aid of ampule files. In the absence of such files, chips of glazed porcelain obtained from broken crucibles or evaporating dishes have proved themselves to be an entirely adequate substitute. Of lesser practical value are glass cutting knives or Carborundum stones, and the like.

Capillary Pipets. For the introduction or transfer of liquid samples or reagents, small micropipets are freshly prepared each time when needed. A piece of soft-glass tubing of 6-mm. outside diameter is held in the non-luminous flame of a Bunsen wing burner. When it softens it is removed from the flame and with a rotating movement drawn out to a capillary of 1-mm. bore. When cool it is cut off, and with the aid of a microburner the end is drawn out to a finer capillary of about 0.3-mm. bore. The finished pipet should be about 10 cm. long and taper down to about 1-mm. outside diameter. To prevent contamination of the sample with small fragments of glass, the end must be fire-polished by holding it briefly in a small flame and blowing air through at the same time, so that the walls do not collapse. A rubber bulb is placed over the wide end if necessary.[4]

Precision Pipets.[12] Such pipets are calibrated for 0.1, 0.2, 0.5 or 1 ml., and the manufacturers enclose a calibration certificate for capacity. Since the volume is known, the specific gravity of the liquid can be determined with the same pipet. The inside diameter of the capillary at the tip and in the upper portion is sufficiently narrow to prevent the liquid from running out if the pipet is held in a vertical position. The pipet is filled by aspirating the liquid from a dish or beaker to just above the calibration mark. The pipet is then held in a horizontal position and any liquid on the outside of the conical tip is wiped off. A piece of filter paper is applied to the outlet and the excess liquid in the pipet is removed until the meniscus of the solution reaches the calibration mark in the stem. Should the mark be missed in withdrawing the excess, dipping of the pipet into the solution to be analyzed will fill it again, provided that the liquid has not receded into the bulb. The solution is transferred by blowing it out of the pipet, care being taken during this operation that the liquid does not spatter in the receptacle. To prevent contamination of the solution with saliva, a suitable rubber tubing with a glass mouthpiece, below which is placed a small cotton plug, is attached to the stem of the pipet. The outside of the pipet is rinsed with a few drops of distilled water while still held in the receptacle and then is refilled twice with distilled water; these washings are added to the sample solution. Pipets having a stem wide enough to permit the introduction of the wash liquid from the top have been found very convenient.

Similarly constructed pipets of 15-cm. total length with 0.5- and 0.1-ml. capacity respectively, but without the elongation in the upper stem and with a ground-glass cap at the tip of delivery stem, are used as standard pycnometers for taking the specific gravity of liquids.[9]

Centrifuge Cones. Centrifuge cones [2, 4] of various sizes (2-, 1-, 0.5-ml., and less capacity), and made either of soft or hard glass or quartz, are used in a number of operations in quantitative organic microanalysis. Centrifuge cones with detachable ground-glass tips [3] have been described and are used in a number of procedures. If loss of substance due to transfer to a standard weighing vessel has to be avoided, the small tip of the centrifuge cone is weighed together with the dry sample and introduced into the combustion apparatus, and at the end of the determination the cone is weighed back.

Wash Bottles. Various types have been described. The wash bottle found most satisfactory (Fig. 6) is of Pyrex glass and consists of a flat-bottom flask with a capacity of 250 and 150 ml., respectively.[16] The head of the flask, which has a 19-mm. inner and 21-mm. outer diameter, carries a standard female ground-glass joint. The upper part of the wash bottle is provided with a standard male ground-glass joint, thus forming an outside glass-to-glass connection with the head, thereby lessening the danger of contaminating the wash liquid with the lubricant or dirt. The two parts of the wash bottle are firmly held together by means of two wire springs attached to glass hooks fused to the head and neck of the flask. The delivery tube extends almost to the bottom of the stand flask, and its nozzle is drawn out to a capillary ending. The mouthpiece is provided with a saliva trap.

If the amount of wash liquid has to be measured, a graduated wash cylinder illustrated in Fig. 7 (Pyrex type 1112), of 50 ml. capacity and with graduations of 1 ml., is very useful. This wash cylinder also is provided with an outside ground-glass joint and has a mouthpiece with a saliva trap like the wash bottle described above.

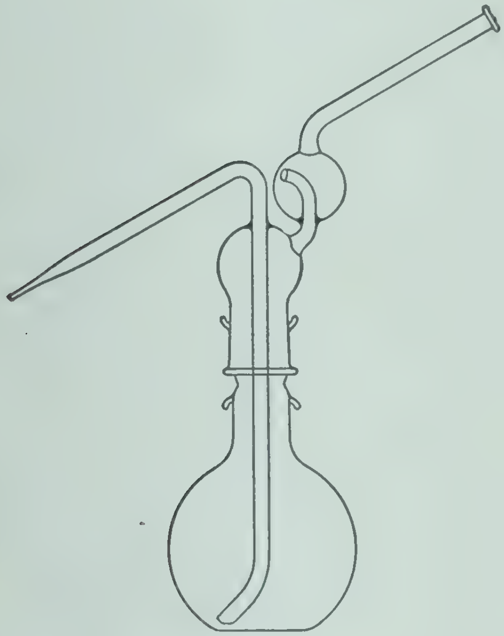


Fig. 6. Wash Bottle.

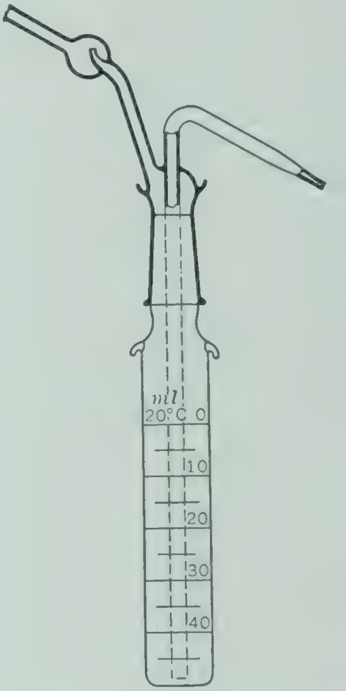


Fig. 7. Graduated Wash Cylinder.

Miscellaneous. Other microanalytical laboratory utensils finding application in microanalytical procedures include a heating apparatus for the protective evaporation, or concentration, of solutions,[6] removable burners,[8] sublimation apparatus,[14] glass knife sharpening blocks,[1] several stands serving as supports for the weighing tubes, etc., and other less common utensils.[15]

LITERATURE

1. Alber, H. K., *Mikrochemie*, **18**, 95 (1935).
2. Benedetti-Pichler, A. A., and Spikes, W. F., "Introduction to the Microtechnique of Inorganic Qualitative Microanalysis," Microchemical Service, Douglaston, L. I., 1935.

3. Elek, S. D., *Mikrochemie*, **19**, 129 (1935).
4. Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York, 1932.
5. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933.
6. Hecht, F., *Mikrochim. Acta*, **3**, 129 (1938).
7. Kirk, P. L., *Mikrochemie*, **16**, 17 (1934).
8. Knoll, A., and Schukal, J., *Ind. Eng. Chem., Anal. Ed.*, **10**, 348 (1938).
9. Niederl, J. B., Mimeographed Outlines, "Semi-micro Qualitative Organic Analysis," New York University, New York, N. Y., 1937, pp. 20–21.
10. Pavelka, F., *Mikrochemie*, **22**, 247 (1937).
11. Reuter, F., *Mikrochemie*, **21**, 131 (1936).
12. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937.
13. Secareau, S., *Bull. soc. chim.*, (5), **2**, 79 (1933).
14. Soltys, A., *Mikrochemie*, **20**, 122 (1936).
15. Viditz, F. von, *Mikrochim. Acta*, **2**, 209 (1937).
16. Westbrook, D. W., Bell Telephone Research Laboratories, New York, N. Y., private communication.
17. Weygand, C., "Quantitative analytische Mikromethoden der organischen Chemie in vergleichender Darstellung," Akademische Verlagsgesellschaft, Leipzig, 1931.

PREPARATION AND WEIGHING OF A SAMPLE FOR ANALYSIS

General Directions

The object to be weighed must have the same temperature as the inside of the balance case. To attain this equilibrium it is placed beside the balance for 5 to 30 minutes before weighing, the time depending on its composition and size, as described. The side doors of the balance should remain open for at least 10 minutes before the weighing. Air currents inside the balance case, unequal temperatures due to body heat and breath of the operator, or adjacent warm objects may cause temperature changes which must be avoided. Smoking while weighing is absolutely fatal to accuracy. As a general rule it is advisable to keep away from the balance as much as possible between the actual weighings.

Sampling

Primarily, quantitative elementary organic microanalysis was developed for *homogeneous* and *pure* organic compounds, and this manual deals chiefly with such compounds. Micro samples of pure compounds taken for analysis are thus true representative samples of the substance to be analyzed. Hygroscopic substances, which tenaciously retain water or solvent, present a number of difficulties and have to be treated accordingly.

Occasionally it is necessary to obtain a representative micro sample from heterogeneous material. It may be a solution or a mechanical mixture. With solutions a thorough shaking is sufficient to secure a uniform aliquot portion of the substance. In a mechanical mixture, however, special sampling and grinding procedures have to be followed, a final particle size of 0.02 mm. being recommended.[3, 4, 12, 15, 18, 19, 21, 32]

Weighing of the Sample

Weighing Boats. The boat, preferably of platinum, weighs approximately 0.6 to 1 gram and is 15 mm. long over all, 5 mm. wide, and 4 mm. high. Boats of porcelain, quartz, or hard glass are used, especially for the combustion of metal salts or if the substance has to be mixed with corrosive reagents.

The boat is cleaned by boiling it for a few seconds in a Pyrex test tube with dilute nitric acid (1 : 1); most of the adhering acid is drained off by tilting it before it is taken out of the test tube; it is held with a platinum wire hook in the outer cone of a non-luminous flame of a gas burner until it glows bright red. The boat is then put on the metal block of the microdesiccator, which is placed beside the balance case. The platinum boat may be weighed immediately afterwards; porcelain, quartz, or hard-glass boats are allowed to stand for 10 minutes to establish temperature equilibrium. During the cleaning of the boat, the desiccator must be kept away from heat.

In the weighing procedure the boat is transferred to the left-hand pan of the balance with the ivory-tipped forceps, and the counterpoise is placed with the same forceps on the right-hand pan. The doors of the balance are then closed.

The boat is weighed as previously described on p. 26. After its weight has been determined, as represented by the beam reading, plus or minus the deflection sum, it is removed from the pan with the aid of the ivory-tipped forceps and placed on a clean surface such as the glass cover of the microdesiccator, or on a sheet of paper. Then the suitable amount * of substance is placed in the boat with the microspatula, and the boat, before being returned to the balance pan, is brushed in order to free it from particles of substance which might cling to its outer sides. The weight of the boat plus the sample is determined like the weight of the empty boat. By subtracting the weight of the empty boat from this result the weight of the sample is obtained. The boat containing the sample is then transferred to the microdesiccator where it remains until the beginning of the actual determination.

The weighing procedure described above should be completed within ten minutes, and since an appreciable change in the zero reading is unlikely to occur during this interval the zero reading need not be determined. But if the boat has to be weighed again after a greater lapse of time, then the zero reading must be determined before and after, and any changes should be taken into consideration and corrected for if they exceed the limits of the weighing error. When weighing samples of 1 mg. or less, an unusually low amount, it is advisable to take the zero reading before and after each weighing and to calculate and apply a possible change according to the instructions given on p. 17. The same applies to oversensitive balances.[6]

---

* The amount of substance to be taken depends upon whether the micro or ordinary analytical balance is employed and to some extent also upon the particular determination, as well as upon the percentage element present in the compound. To be certain that the weighing error is well within the allowable experimental error the approximate amount required should be calculated beforehand. See p. 6.

Occasionally the sample has to be mixed with a solid oxidizing reagent, such as potassium bichromate for instance, which is used in the determination of carbon and hydrogen in organic salts, or with copper oxide if the compound is explosive or difficultly combustible. In this case the previously dried and finely powdered reagent is added to the weighed sample and mixed with it with the aid of a short platinum wire which is left in the boat during the course of the determination.

Weighing cups of tin or aluminum foil, which are made by taking a piece of foil 2 cm. square, folding it over the end of a glass rod 5 to 7 mm. in diameter, and then cutting it to a height of about 5 mm., find extensive application in a number of determinations.[14, 23, 26, 34]

Weighing Tube (Fig. 8). This weighing tube, also referred to as charging tube, [1, 9, 11, 16, 17, 24, 26, 35] is used whenever a solid substance is weighed which afterwards has to be transferred to another vessel such as an Erlenmeyer titration flask or a Kjeldahl digestion flask. It consists of a soft-glass tube of about 2-cm. length and 4-mm. inner diameter, to which is fused a glass rod about 11 cm. long and 1.5 mm. thick. It can be readily made in the laboratory by drawing out a soft-glass tube to a capillary of 4-mm. inner diameter, then drawing out this capillary once more in the flame of a microburner to a finer capillary, which is broken off 3 to 5 mm. beyond the constriction and fused to form a small knob. A glass rod about 1.5 mm. thick is sealed to this knob and cut to form a stem about 11 cm. long. The tube is cut to a length of 2 cm. and its mouth fire-polished, but without permitting its rim to curve inward. The weighing tube is counterpoised with a short glass rod in the following manner: A glass rod of about 4-mm. diameter is drawn out at one end to a thickness of about 1 mm. and the heavy part of the glass rod is cut off approximately 1 cm. beyond the beginning of the fine glass rod. The weighing tube is then placed on an analytical balance and small pieces from the thin end of the counterpoise are cut

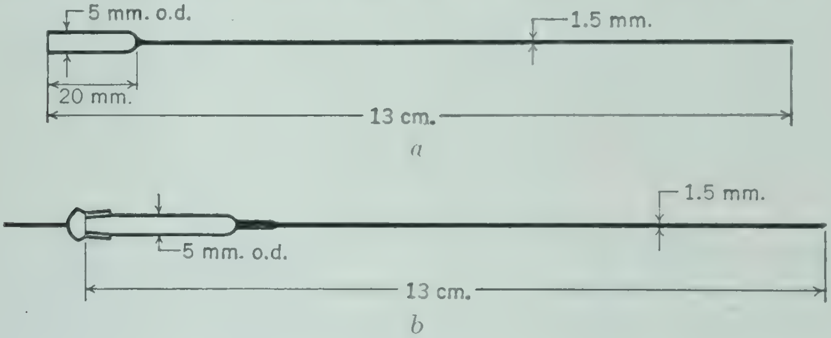


FIG. 8. *a*, Weighing Tube; *b*, Weighing Tube with Ground-Glass Cap.

off until its weight approaches that of the weighing tube but is still about 1 to 2 mg. greater. The final adjustment is carried out by placing the weighing tube on the hooks of the left pan and the counterpoise on the right pan and filing off the thick end of the counterpoise until it is approximately 1 mg. less than the weight of the tube. The blunt end of the counterpoise is then fire-polished, and what is left of the fine glass rod at the other end is bent by holding it in a flame, so that it will take a convenient shape enabling it to be held by the forceps when it is transferred to and from the balance.

In glass vessels, constancy of weight is somewhat more difficult to obtain and frequently can be achieved only after the glass object has been in the balance for ten or more minutes and until equilibrium in temperature has been reached.

The weighing tube is moved to and from the balance with the aid of a wire fork; before weighing it is wiped clean with a chamois and thereafter is handled only with protected fingers. The substance to be analyzed is introduced into the weighing tube with the microspatula, after which the surface and the orifice of the tube are brushed off. When the tube has been weighed it is removed from the hooks of the left-hand pan with the wire fork, grasped at its lower end with a chamois, and the contents emptied into whatever vessel is used for a particular determination. By tapping the stem of the weighing tube while it is inserted in the receptacle, most of the substance will be transferred. The tube, without brushing, is then weighed back. The weight of the substance is the difference between the two weighings

Weighing Cups and Bottles. A variety of flat-bottomed weighing cups and bottles are used in quantitative organic microanalysis.[8, 10] They may and often should be provided with ground-glass stoppers, and their size as well as shape are determined by the purpose which they serve. They are principally used to weigh very viscous, syrupy or hygroscopic liquids, or semi-solids subjected to the micro Carius halogen or sulfur determination and for some of the structure analytical methods such as the alkoxyl, alkimide, and acyl determinations.[8]

A variety of weighing capillaries are in use in quantitative microanalytical work, depending on the method of analysis employed.

Capillaries for the Various Wet Combustion Methods. These are made of hard glass 1 to 1.5 mm. wide and 5 cm. long, open at both ends. They are weighed on a small support of metal sheet or wire.[11, 26, 33, 35] The weighed capillary is dipped into the substance to be analyzed which has previously been placed on a glass slide. About 3 to 5 mg. of the liquid substance will thus enter the capillary. A solid sample is pressed into the capillary, or it may have to be pushed into it with a glass thread fitting tightly into the bore. The capillary is brushed and wiped and placed on the balance pan so that the end containing the sample protrudes over the edge of the pan. After 5 minutes the weight of the capillary plus the sample is determined and the capillary dropped into the reaction vessel. This method of weighing has to be used for reactive substances or for semi-solids, waxes, oils, syrups, etc., which are to be subjected to wet combustion procedures (pp. 151, 182, 200).

Capillaries for the Vaporimetric Molecular-Weight Determination [29] (p. 221). Capillaries 8 to 9 cm. long and not less than 1.5 mm. in inside diameter are made from soft glass. They weigh about 150 to 200 mg. and are counterbalanced either by similar capillaries or by the weights and weighed as usual. The substance is melted on a microscope slide, on a small watch glass, or in a wider capillary. Then the weighed capillary is dipped into the melted substance which by capillary attraction is allowed to rise to a height of not more than 4 mm., thus giving an average sample of 3 to 6 mg. The capillary is then withdrawn and the sample allowed to cool and solidify. If solidification does not take place, a fragment of the original crystalline sample is brought in contact with the liquid to induce crystallization. The outside is again cleaned with a chamois, the capillary placed on the hooks of the left pan, and weighed after 5 minutes.

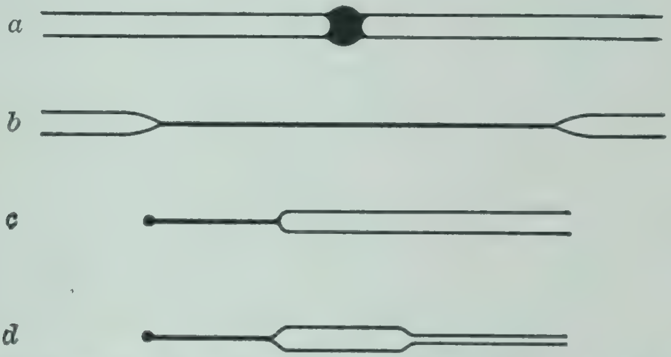


FIG. 9. Weighing Pipet. *a*, Capillary Fused in the Center; *b*, Drawn out to a Glass Thread; *c*, Sealed at One End; *d*, Finished Weighing Pipet.

In addition to the aforementioned glass weighing vessels for solid substances, a number of modifications and combinations have been described.[5, 7, 24, 30, 31] Any of these may be used or varied to suit individual requirements.

Weighing Pipets (Fig. 9).[22, 26, 30] A soft-glass tubing of 6-mm. outside diameter is heated to its softening point in the non-luminous flame of a gas burner. Then it is removed from the flame and with a circular movement around its axis it is drawn out to a capillary of 1-mm. bore, which is cut into pieces of 10-cm. length. The center of such a capillary is held in the flame of a microburner with constant turning until the walls collapse and form a solid thread which is drawn out to a diameter of about 0.5 mm. and to a length of approximately 3 cm. This thread is heated again in the center until it separates into two parts, thereby forming two capillaries, each possessing a convenient handle, the end of which is dipped into the flame until it forms a small knob. Since the capillary still is fully open at the other end, chemical reagents may now be introduced. For most combustion procedures, such as the determination of nitrogen by the Dumas method, the carbon-hydrogen, and in some of the other determinations, a few small crystals of anhydrous potassium chlorate are inserted in the capillary, centrifuged down to the sealed end, and fixed by fusion.

The capillary is then heated in the microflame approximately 10 mm. from the sealed end and drawn out to a fine capillary of 0.1-mm. bore, thus forming the bulb and the delivery stem of the weighing pipet. In the vaporimetric molecular-weight determination [22] the bulb of the pipet is much shorter, only 3 to 5 mm., but its diameter is greater, about 2 mm. The fine delivery stem is cut to a length of 10 to 15 mm., care being taken at the same time that the capillary is open. It is advisable to prepare a number of such pipets at one time and keep them in a desiccator over a dehydrating agent.

The pipet is wiped before being weighed, is placed on the metal block of the microdesiccator, and after a few minutes its weight is determined. Then a small amount of the sample to be analyzed is placed on a slide or watch glass, the bulb of the capillary is gently warmed in the flame of a microburner, and the fine end of the delivery stem dipped quickly into the liquid. By subsequent cooling, a vacuum is created inside the bulb which causes the liquid to be drawn in. The droplet thus introduced is centrifuged to the bottom of the bulb so that as little substance as possible remains in the upper part of the pipet. Finally, the end of the delivery stem is sealed by holding it for a moment in the outer cone of a flame; the outside of the capillary is wiped clean and the pipet reweighed.

The above general procedures may be suitably modified; for very volatile liquids, for instance, the delivery stem of the pipet is made longer and finer, and the substance is sufficiently cooled prior to introduction into the pipet. For high-boiling liquids, the finished weighing pipets but with a wider delivery stem can be employed, or else the liquid may be introduced by means of another capillary before the stem is made, in which event the capillary delivery stem must be drawn out and sealed off without loss of glass.

For viscous and high-boiling liquids a *straight capillary* 9 to 10 cm. long is used. It is weighed as usual. One of its ends is dipped into the liquid, its outside wiped clean, and the capillary reweighed. It is cut about 2 mm. above the surface of the liquid and put into the reaction vessel or combustion tube. For low-melting solids such as fats and waxes, or for semi-solids, similar procedures may be employed (p. 46).

Many other weighing implements have been recommended for special applications, such as long capillaries of various diameters, combustion capillaries, etc., [5, 11, 26, 30, 35] as well as transfer capillaries or pipets of various lengths and diameters, but it is beyond the scope of this book to discuss them all in detail.

Hygroscopic Material

Although the many intricate devices and procedures adapted for the treatment of various hygroscopic substances [2, 7, 11, 13, 20, 25–29, 31, 34, 35] cannot be taken up in detail, a few general procedures are given below which usually involve (*a*) the drying of the sample and (*b*) the protection of the dried material from atmospheric moisture during the weighing period.

Drying. For this purpose it is generally sufficient to place the substance in a vacuum desiccator containing appropriate drying agents—such as anhydrone, concentrated sulfuric acid, phosphorus pentoxide—and resort to evacuation. Since the sample is small, the drying is, as a rule, quickly accomplished. The air entering the desiccator during the re-establishment of the normal pressure should be dried with the same drying agent.

For substances which have to be treated at higher temperatures but at normal pressure, the customary drying ovens will suffice; special metal blocks, heated with gas or electricity, are an added convenience.[9, 11, 26]

Occasionally it is necessary to dry the sample at higher temperatures either at atmospheric or reduced pressure. Drying pistols of the usual design which may be evacuated and are charged with a drying agent serve this purpose very well.[27, 34] A microvacuum desiccator as shown in Fig. 10 [9, 11, 26, 35] may also be employed; it consists of a glass tube 24 cm. long and 1 cm. in outside diameter, which is separated into two parts (*a* and *b*) by a capillary tubing 3 cm. long. The front section (*b*) is filled with anhydrone; cotton wads are placed at both ends to prevent the capillaries from becoming clogged. Part (*b*) is closed with a perforated rubber stopper carrying capillary (*c*), which is connected to a bubble counter for the purpose of indicating a stoppage of the air current.[7] The platinum or porcelain boat containing the sample is placed in the drying chamber (*a*) which is closed with a drying tube (*d*) inserted in a rubber stopper. This drying tube has the same dimensions as (*b*) and is connected to a vacuum pump or water pump with a capillary tubing. After the minimum pressure has been obtained the entire desiccator is placed in the wide groove of the heating block, as shown in the illustration, and held in position by two cylindrical corks which are flattened at the bottom so that the apparatus can be placed on the table without rolling over and thereby spilling the sample. The block is heated to about 70° C. for one-half to one hour, which usually suffices; then the desiccator is removed from the heating block and permitted to cool. The vacuum is broken gradually, and after the drying tube is detached the boat containing the substance is transferred immediately to the glass-stoppered *weighing piggy* described below and weighed.

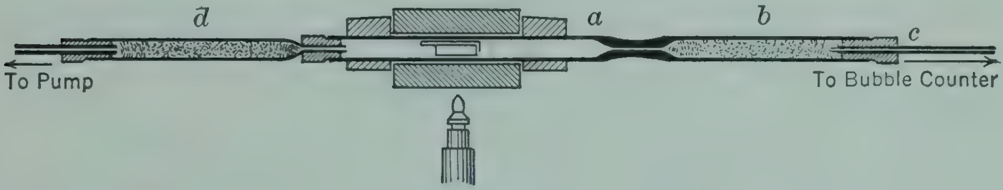


FIG. 10. Micro Vacuum Desiccator. Explanation in Text.

Weighing.[2, 11, 20, 25, 26, 28, 35] Only material which shows adequate constancy of weight, that is, agreement of several weighings within ±5 micrograms, can be used for quantitative organic microanalysis if interpretability of the results is expected. The weighing of the previously dried material is best carried out in a *weighing piggy,*[2, 22, 26] illustrated in Fig. 11. This device is equipped with a well-fitting ground-glass stopper provided with a long handle. The inside must be wide enough to permit the insertion of the boat; it rests on two short legs, and it has a glass handle at the end. Before being weighed it must be wiped with a chamois like the various other glass vessels described before. A weighing tube having a ground-glass cap may also be used for the weighing of hygroscopic substances.

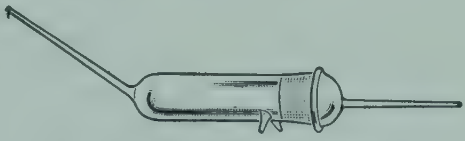


FIG. 11. Weighing "Piggy."

LITERATURE

1. ABRAHAMCZIK, E., and BLUEMEL, F., *Mikrochemie*, **24**, 268 (1938).
2. ALBER, H. K., *Mikrochemie*, **25**, 47, 167 (1938).
3. BAULE, B., and BENEDETTI-PICHLER, A. A., *Z. anal. Chem.*, **74**, 442 (1928).
4. BENEDETTI-PICHLER, A. A., *Z. anal. Chem.*, **61**, 305 (1922).
5. BENEDETTI-PICHLER, A. A., and SPIKES, W. F., *Mikrochemie*, **15**, 284 (1934).

6. Breuer, F., *Ind. Eng. Chem., Anal. Ed.*, **9**, 354 (1937).
7. Britzke, E. V., and Hoffmann, E., *Mikrochemie*, **22**, 121 (1937).
8. Elek, A., Rockefeller Institute for Medical Research, New York, N. Y., private communication.
9. Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York, 1932, pp. 62–66.
10. Ferguson, G. E., and Scheflan, L., *Ind. Eng. Chem., Anal. Ed.*, **12**, 553 (1940).
11. Friedrich, A., "Die Praxis der quantitativen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933.
12. Grünsteidl, E., *Mikrochemie*, **16**, 247 (1935).
13. Hayman, D. F., Rochester Meeting, Am. Chem. Soc., September, 1937; *Ind. Eng. Chem., Anal. Ed.*, **4**, 256 (1932), **8**, 342 (1936); **10**, 55 (1938); *Mikrochemie*, **23**, 238 (1937).
14. Hernler, F., *Mikrochemie, Pregl Festschrift*, 1929, p. 14.
15. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **5**, 363 (1933); **7**, 294 (1935).
16. Lieb, H., and Krainick, H., *Mikrochemie*, **9**, 367 (1931).
17. Lindner, J., "Mikromassanalytische Bestimmung des Kohlenstoffes und Wasserstoffes mit grundlegender Behandlung der Fehlerquellen in der Elementaranalyse," Verlag Chemie, Berlin, 1935.
18. Lucas R., and Grassner, F., *Mikrochemie*, **6**, 129 (1928).
19. Mika, J., *Z. anal. Chem.*, **73**, 257 (1928).
20. Milner, R. T., and Sherman, M. S., *Ind. Eng. Chem., Anal. Ed.*, **8**, 427 (1936).
21. Müller, E., and Willenberg, H., *J. prakt. Chem.*, **99**, 34 (1929).
22. Niederl, J. B., Trautz, O., and Plentl, A., *Ind. Eng. Chem., Anal. Ed.*, **8**, 252 (1936).
23. Niederl, J. B., and Whitman, J. B., *Mikrochemie*, **11**, 287 (1932).
24. Pirsch, J., *Ber.*, **65**, 862 (1932).
25. Rodden, C. J., *Ind. Eng. Chem., Anal. Ed.*, **11**, 405 (1939).
26. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937.
27. Roth, H., and Daw, E. B., *op. cit.*, p. 55.
28. Sherman, M., and Milner, R. T., Rochester Meeting, Am. Chem. Soc., September, 1937.
29. Shiwow, W. C., *Betriebs Lab.* (*Russ.*), **4**, 824 (1935).
30. Soltys, A., *see* Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1927, p. 242; *Mikrochemie, Molisch Festschrift*, 1937, p. 393.
31. Sternberg, H., *Mikrochemie*, **22**, 187 (1937).
32. Strebinger, R., and Radlberger, L., *Österr. Chem. Ztg.*, **22**, 67 (1919).
33. Tiedcke, C., *Mikrochemie*, **16**, 171 (1935); Rochester Meeting, Am. Chem. Soc., September, 1937.
34. Unterzaucher, J., *Mikrochemie*, **18**, 315 (1935).
35. Weygand, C., "Quantitative analytische Mikromethoden der organischen Chemie in vergleichender Darstellung," Akademische Verlagsgesellschaft, Leipzig, 1931.

STANDARD SOLUTIONS

Principle

Three 0.01 *N* standard solutions are necessary:

0.01 *N* potassium biiodate solution
0.01 *N* sodium hydroxide solution
0.01 *N* sodium thiosulfate solution

These solutions are sufficient for the procedures involving alkalimetric, acidimetric and iodometric titrations.[9, 42] These titrations proceed according to the following equations:

(*a*) In alkalimetric and acidimetric titrations:

$$KH(IO_3)_2 + NaOH = NaIO_3 + KIO_3 + H_2O$$

(*b*) In iodometric titrations:

$$KH(IO_3)_2 + 10KI + 11HCl = 11KCl + 6H_2O + 6I_2$$

$$12Na_2S_2O_3 + 6I_2 = 12NaI + 6Na_2S_4O_6$$

Apparatus

Microburet (Fig. 12). The microburet consists of the vertical stem (*a*), the horizontal delivery tube (*b*), and the filling tube (*c*) which is provided with an automatic zero-point filling arrangement. The middle part consists of the calibration column (*d*), which is approximately 33 cm. long and has a capacity of 10 ml. with subdivisions of 0.05 ml. The lower part fits into a supply bottle of 1-liter capacity by means of a ground-glass joint and extends almost to the bottom of the supply bottle (*e*). Somewhat above the ground-glass joint a glass tube carrying a stopcock is sealed to the stem and a piece of rubber tubing which is dissected by a T-tube connects this glass tube to the pumping device (*f*). About 4 cm. above the ground-glass joint the vertical filling tube (*c*) branches off. On the opposite side of this tube is attached the nearly horizontal delivery tube (*b*) which extends approximately 10 cm. forward and then a short distance downward. To this end is attached a piece of good seamless rubber tubing 5 cm. long containing a glass bead which serves as a seal as well as a delivery valve when it is pressed

OOD TECHNOLOGICAL RESEARCH INS
LIBRARY

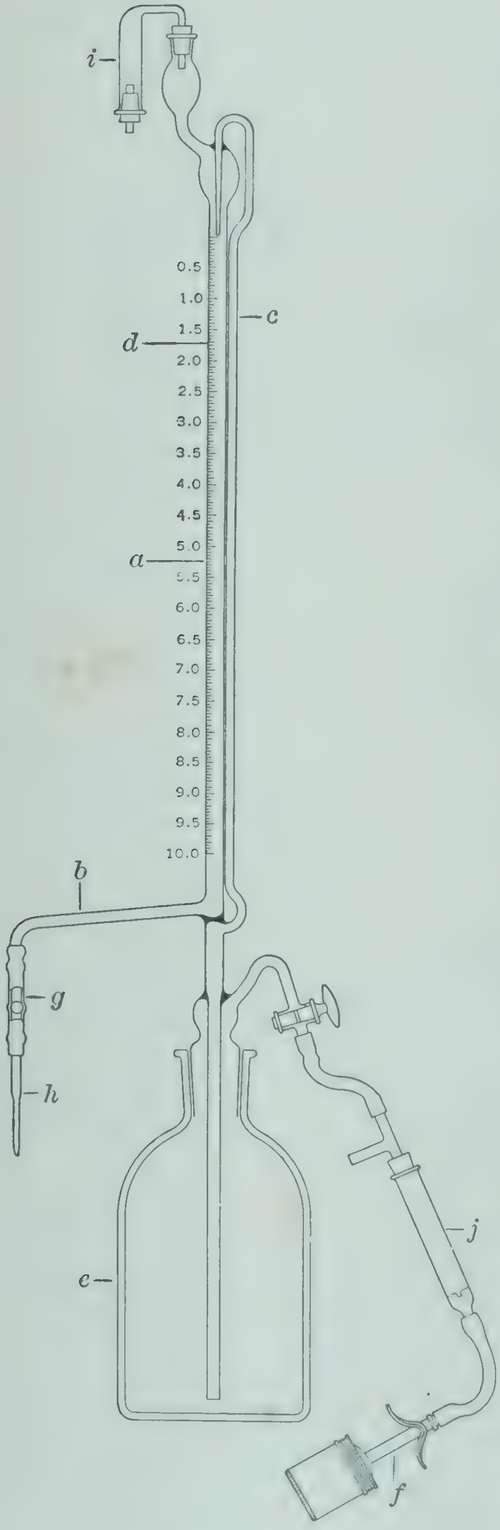


FIG. 12. Microburet.

with the fingers. To this rubber tubing (*g*) is connected the capillary delivery tube (*h*) which is approximately 7 cm. long and drawn out to a fine capillary ending of about 2-cm. length and a fine enough inner diameter at the tip to permit the withdrawal of drops measuring 0.01 ml.

To protect the solution from carbon dioxide, a safety tube (*i*), filled with Ascarite, is attached to the opening on top of the buret, and another safety tube (*j*), filled with the same reagent, is placed between the pumping device and the T-tube.

The buret as well as the supply bottle is cleaned by washing it repeatedly with cleaning solution, and then with ammonia and soap solution, after which it is exhaustively rinsed with distilled water. Before being filled the buret should be rinsed twice with small amounts of the respective standard solution.

Buret Stand. Although no specific device is necessary, the stand described below has proved to be a convenient arrangement.[6] This stand has as a base a metal plate measuring 40 by 13.5 cm., on the four corners of which the vertical supports for the top plate are mounted. These supports are 11 cm. in height and 12 mm. in diameter. They are hollow and cylindrical in shape and support the top plate which is identical in dimensions to the base. The top plate has three circular openings, 11 cm. in diameter, for the insertion of the supply bottles of three microburets. Held to the top plate by means of a vertical metal flap 5 mm. wide is the vertical front plate, measuring 40 by 10.5 cm. and made of white transparent glass. The horizontal frontal titration plate, measuring 40 by 9 cm., is made of the same material. Electric lighting units with *daylight* bulbs (blue, frosted lamps) are either built in between the burets or placed close to the burets by attaching them to the usual laboratory stand.

Dropping Bottles. Glass-stoppered dropping bottles of approximately 50-ml. capacity are used as containers for the indicator solutions.

Titration Flasks. Pyrex or quartz Erlenmeyer flasks of 25- and 50-ml. capacity are required; they must be steamed out before every titration.

Steaming Apparatus (Fig. 13). This apparatus consists of a round-bottomed Pyrex flask of 1-liter capacity. A conical glass funnel of 60° angle and having a diameter of 8.5 cm. at the rim is inserted into the neck of the flask by means of a one-hole rubber stopper. The stem of the funnel, 8 cm. long and 5 mm. in diameter, continues vertically up into the funnel for 12 cm. At its base the funnel is provided with a descending drain tube 4 cm. long and 6 mm. wide to which a rubber tubing of suitable length is attached to permit the condensing steam to drain into a sink. The steam which is generated in the round-bottomed flask passes through the funnel tube and by placing a titration flask over the funnel tube as shown in the illustration the flask is effectively treated with steam.

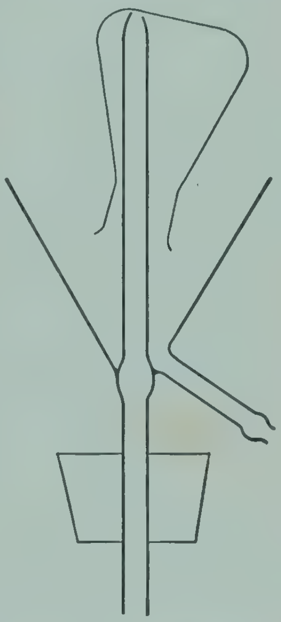


Fig. 13. Steaming Apparatus.

Reagents

Potassium Biiodate. Preparation:[49] 110 grams of potassium chlorate is dissolved in 450 ml. of warm water and 40 ml. concentrated hydrochloric acid contained in a 2-liter Erlenmeyer flask which is placed in a well-ventilated hood. One hundred grams of powdered c.p. iodine is then added, and the reaction mixture is slowly warmed with occasional shaking until the reaction commences. The reaction proceeds rather violently with the evolution of chlorine, but if the heating is slight very little iodine is lost. After the reaction subsides the solution is boiled for a few minutes and filtered while hot. On cooling about 150 grams of biiodate crystallizes out, and this material is about 90%

pure. The crystals are filtered off by suction and are redissolved in 3 to 4 times their weight of boiling water, the resulting solution being filtered while still hot. After standing in the refrigerator over night the crop of crystals obtained is filtered off and dried at 100° C. in a drying oven for about one hour. The yield is about 100 grams of salt which is of sufficient purity.

Sodium Carbonate. Several grams of C.P. halogen and sulfate-free sodium carbonate is dried in a porcelain crucible at 300° C. for one hour with the thermometer in contact with the substance. Then, while still warm, the substance is transferred to a desiccator containing phosphorus pentoxide.

Sodium Hydroxide. C.P., in pellets or sticks.

Sodium Thiosulfate Pentahydrate (C.P. crystals).

Sodium Chloride. C.P. reagent quality.

Potassium Iodide Solution (4%). Four grams of potassium iodide C.P. is dissolved in 100 ml. of distilled water.

Hydrochloric Acid (sp. gr. 1.18).

Sulfuric Acid[9] (Reagent quality). Ten per cent aqueous solution.

INDICATOR SOLUTIONS

Phenolphthalein. One per cent soluton in 95% ethyl alcohol.

Methyl Red (0.3%). This solution is prepared by treating 0.15 gram of methyl red (*p*-dimethylaminoazobenzene-dicarboxylic acid) with 40 ml. of 0.01 *N* sodium hydroxide solution. The mixture is filtered and the filtrate stored in a glass-stoppered dropping bottle. The capillary of the glass stopper is drawn out to a solid glass rod of about 1-mm. thickness. Since only a trace of this indicator solution need be used, the glass rod is merely dipped into the indicator solution and some of it is transferred to the titration vessel by touching the inner wall. By tilting the titration flask the indicator is mixed with the solution to be titrated.

Starch Solution[9] (1%). One gram of water-soluble starch is triturated with a few milliliters of cold distilled water, the resulting suspension is added to a boiling 20% aqueous salt solution, boiled for 5 minutes, allowed to cool, and then filtered. The filtered solution is transferred to a dropping bottle.

0.01 *N* POTASSIUM BIIODATE SOLUTION[9]

Preparation. 3.8994 grams of pure $KH(IO_3)_2$ is weighed on an ordinary analytical balance in a small porcelain dish which has previously been counterpoised and weighed. The substance is then transferred to a 1-liter volumetric flask. To avoid loss of substance, it is best to place the porcelain dish containing the potassium biiodate in a wide funnel which is inserted into the neck of the volumetric flask. Hot distilled water is sprayed over the funnel and the substance, which thereby is washed into the volumetric flask. The empty dish is thoroughly rinsed, and the flask is filled to about three-fourths of its capacity with distilled water previously boiled to remove carbon dioxide. In order to dissolve the potassium biiodate completely, the flask may be heated on a steam bath. Thereafter the solution is cooled to the temperature at which the volumetric flask is calibrated and the flask filled up to the mark. Finally, the solution is transferred to the supply bottle of the microburet which previously has been cleaned as well as rinsed with two small portions of the solution.

Standardization. Although normally it is not necessary to standardize the solution when prepared as described above, its strength may be checked occasionally by titration with sodium carbonate as follows: A 3- to 5-mg. sample of pure sodium carbonate, previously dried at 300° C., is weighed in a stoppered weighing tube, transferred to a steamed-out 50-ml. Erlenmeyer flask, and dissolved in 5 ml. of distilled water. One drop of phenolphthalein solution is used as indicator, and the solution is kept at boiling temperature during the titration.

0.01 *N* Hydrochloric Acid Solution

This solution may be employed instead of the above potassium biiodate solution.

Preparation. There are several ways of preparing 0.01 *N* hydrochloric acid. One is by diluting a given volume (100 ml.) of 0.1 *N* hydrochloric acid with nine times its volume of carbon-dioxide-free distilled water (900 ml. to give 1 liter of 0.01 *N* hydrochloric acid) using a suitable (1-liter) volumetric flask. The 0.1 *N* hydrochloric acid solution may be prepared from redistilled concentrated hydrochloric acid in the usual manner, or by the use of Fixanal "Acid hydrochloric," which has proved entirely satisfactory. A tube of 0.1 *N* equivalent hydrochloric acid is pierced with the Fixanal standard piercing apparatus and the solution transferred quantitatively to a 1-liter volumetric flask. The transfer is accomplished by placing the delivery tube of the piercing apparatus which contains the glass nail in a ring clamp attached to a stand. The outlet of this delivery tube is inserted in the neck of the volumetric flask. Both are previously cleaned with cleaning solution and repeatedly rinsed with distilled water. The outside of the Fixanal tube is also rinsed repeatedly with distilled water. The tube is then thrown down into the delivery tube, so that the indentation at the lower end is pierced by the glass nail of the piercing apparatus. The upper indentation is pierced with another glass nail and the inside of the Fixanal tube is rinsed profusely with distilled water, which is introduced through the upper opening.

For filling the volumetric flask as well as for the rinsing of the Fixanal tube distilled water is employed which is again boiled out shortly before being used. While permitted to cool, the flask is protected from the carbon dioxide in the air by an Ascarite tube. The solution is standardized in exactly the same manner as the potassium biiodate solution.

0.01 *N* Sodium Hydroxide Solution

Preparation.[50] A concentrated, carbonate-free solution is prepared by dissolving C.P. sodium hydroxide in an equal weight of distilled water in a rubber-stoppered flask which is placed on a water bath and kept at about 80° C. for twelve hours or at room temperature for at least a month. After this time any carbonate present will have precipitated out and settled to the bottom of the flask. One milliliter of the supernatant clear solution contains approximately 0.79 gram of sodium hydroxide. One half milliliter of this solution is added to 900 ml. of freshly boiled distilled water contained in a 1-liter volumetric flask. After thorough mixing, 5 ml. of this solution is withdrawn by means of a pipet and is titrated against the 0.01 *N* potassium biiodate solution using methyl red or phenolphthalein as the indicator. The additional volume of distilled water needed is calculated, but only 90% of the theoretical amount required is actually added. The titration and addition of distilled water are repeated until the strength of the sodium hydroxide solution is within 0.0101 and 0.0099 *N*. Then the solution is transferred to the supply bottle of the microburet.

Standardization. Seven to eight milliliters of the 0.01 *N* potassium biiodate solution, accurately measured, is run into an Erlenmeyer flask and a drop of phenolphthalein indicator solution is added. The solution is titrated with the sodium hydroxide solution, care being taken to remove any carbon dioxide from the titration solution by boiling it just before it turns alkaline. The titration is repeated using methyl red indicator solution. The end points obtained with the two above-mentioned indicators may differ slightly.

0.01 *N* Sodium Thiosulfate Solution

Preparation. 2.48 grams of sodium thiosulfate pentahydrate crystals is weighed on an ordinary analytical balance and quantitatively transferred to a 1-liter volumetric flask, the procedure being similar to the preparation of the 0.01 *N* potassium biiodate solution, but cold distilled water being used for rinsing. Next, 1.5 ml. of amyl alcohol is introduced and the flask is filled to the mark with distilled water. The solution is then ready for transference to the supply bottle of the microburet.

Standardization. The 0.01 *N* potassium biiodate solution becomes 0.12 *N* when reacting with potassium iodide in acid solution. Thus 0.7 to 0.8 ml. of the 0.01 *N* potassium biiodate solution is accurately measured with the microburet and run into a glass-stoppered 125-ml. Erlenmeyer flask. Three milliliters of 10% sulfuric acid[9] in the cold, or concentrated hydrochloric acid with heating, and 2 ml. of the 4% potassium iodide solution are added. After the solution has stood for two minutes, titration with the sodium thiosulfate solution is begun. Before the complete disappearance of the iodine color the solution is diluted with 10 ml. of distilled water to about 20 ml. total volume; four or five drops of the starch indicator solution is then added and the titration stopped as soon as the blue color changes to a faint pink coloration.

Remarks

Standard Solutions and Indicators. The usability of the 0.01 *N* potassium biiodate solution in alkalimetric and acidimetric titrations encountered in quantitative organic microanalysis was investigated by J. B. Niederl and co-workers[42] and for iodometric titrations by A. Elek.[9] It was found that there was no interference (oxidation, etc.) when this reagent was used instead of the hydrochloric acid formerly employed. The use of solid samples of potassium biiodate in standardizations has long been established.[9, 13, 28, 29, 49] Although this substance is in itself a standard, its 0.01 *N* solutions, which have proved to be very stable, may be checked in a similar manner as any other 0.01 *N* acid solution by titrating it with sodium carbonate[35, 50] which has been dried according to G. Lunge.[39]

The stability of the 0.01 *N* sodium hydroxide and the 0.01 *N* sodium thiosulfate solution has been repeatedly investigated,[1, 2, 27, 30, 37, 42, 44, 46] and it has been found advisable to check the factor of the 0.01 *N* sodium thiosulfate solution at least once a week,[16] while a monthly check of the 0.01 *N* sodium hydroxide solution is usually sufficient. For the stabilization of the sodium thiosulfate solution the addition of a small amount of amyl alcohol (0.15%) or of sodium carbonate (0.02%) has been found adequate.

The use of the three indicators, phenolphthalein for titrating organic acids with sodium hydroxide solution, methyl red for titrating ammonium hydroxide solution, and soluble-starch solution for use in iodometric titrations, is well established. These indicators may be substituted by others having a similar pH range.

Microburets. Many types of microburets have been designed and many modifications have been devised.[3, 4, 5, 17, 22, 31, 32, 40, 43, 51, 53, 54, 56, 58, 59, 60] Essentially any buret consists of three parts: the calibrated column, the outlet, and the filling device. Modifications in regard to the calibrated column are limited to the graduation employed. The burets used for titrations with 0.01 N solutions, as for the milligram processes employed in quantitative organic elementary analysis, are calibrated to 0.05 ml. for a total volume of 10 ml.[13, 42, 44, 46] When finer graduation is necessary, the total capacity is smaller (2.5 ml.[40] and less). Under these conditions *syringe* burets are used.[5, 7, 10, 26, 33, 36, 37, 45, 57] Such types of burets allow the withdrawal of as little as cubic millimeters and fractions thereof. These burets are usually operated with the aid of a mercury seal, the plunger being mounted on a drum with appropriate calibration. Some of these syringe burets even permit titrations on a microscope slide.[21] Somewhat larger burets which are mounted vertically but operate without a mercury seal have also been constructed.[48]

The outlet arrangement of a microburet consists essentially of the capillary tip and provisions for the withdrawal and stoppage of the titration fluid. This may be a rigid and permanent arrangement such as is found in all burets operated by means of a glass stopcock or a pinchcock which may be arranged vertically or horizontally.[2] The stopcock may be situated right below the vertical calibrated column,[14] or it may, as is usual, be located at the delivery tube of the buret. For ease of operation without impairing precision, a glass bead is usually preferred, in which case the side arm of the buret is connected with the capillary tip by means of a rubber tubing. A glass bead is inserted in this rubber tubing which, when pressure is exerted upon the rubber tubing, allows the withdrawal of the solution in amounts small enough to be entirely satisfactory for microchemical practice. The titration flask can be held in one hand while the flow of the fluid is regulated with the other, and an interruption of the titration after withdrawal of each drop which occurs with glass stopcocks is avoided. Such an arrangement, furthermore, allows an easy exchange of the buret tips and of course eliminates the freezing-in of glass stopcocks, as well as clogging and contamination with stopcock grease. Burets with stopcock arrangements both at the top[38] and in the back, or provided with friction capillaries, have been described, as well as burets without any stopcocks.[19, 36, 52] One such vertical buret, with mercury acting as both sealing and replacing liquid, has been devised for potentiometric titrations.[11] Precautions to be observed in calibrating microburets have been given by E. Schilow.[47]

As mentioned above, exchangeable capillary tips are usually preferred.[23] They may be connected to the delivery tube with a short piece of rubber tubing, or may be attached to it by means of a ground-glass joint.[25] To lessen capillary attraction and adhesion of the droplets delivered, these capillary buret tips may be treated with a trace of paraffin.

Various refilling devices are known.[16, 40] The one most widely used is the pumping arrangement.[13, 42, 44, 46] By increasing the air pressure in the system, part of the solution in the supply bottle is forced through the vertical filling tube into the top portion of the buret, which may or may not carry an automatic zero-point adjustment. Usually it is not advisable to rely on an automatic zero-point arrangement on account of possible incomplete drainage and leakage. The filling tube is usually outside and back of the calibrated column of the buret.

Most burets designed for routine work are provided with an adequate supply bottle of sufficient capacity to cope with the particular needs. One-liter supply bottles are usually employed. The smaller burets, the syringe burets, etc., do not need a supply bottle; other burets may have special devices on the top to serve as a reservoir [41] or overflow chamber [40] for refilling them; such devices operate on either the vacuum or pressure principle.

Finally, there exist combinations of macro and microburets,[8, 15, 24, 60] as well as combinations of pipets with burets [16] and double burets.[12, 18] The macro-microburets [8, 15, 24, 60] usually consist of two calibrated columns, one with a larger total capacity but subdivided only in cubic centimeters or multiples thereof, and a second one of lesser capacity with appropriate subdivisions to 0.05 ml. and less. They are connected to the same delivery tube by means of a two-way stopcock. A buret connected to several supply bottles containing different standard solutions also has been reported,[19, 20] as well as burets with automatic (electromagnetic) stirring devices.[34] Conditions and arrangements of apparatus for titrations in vacuum [55] as well as for electrometric titrations [38] have been studied.

LITERATURE

1. Abderhalden, E., "Handbuch der biochemischen Arbeitsmethoden," Urban & Schwarzenberg, Berlin and Vienna, 1910, Vol. I, p. 450.
2. Bang, I., "Mikromethoden zur Blutuntersuchung," Third Ed., J. F. Bergmann, Munich and Wiesbaden, 1922, pp. 8, 19, 33.

3. Benedetti-Pichler, A. A., "Die Fortschritte der Mikrochemie in den Jahren 1915–1926," F. Deuticke, Leipzig and Vienna, 1927, pp. 150–157.
4. Benedetti-Pichler, A. A., *Z. anal. Chem.*, **73**, 200 (1926).
5. Chaney, A. S., *Ind. Eng. Chem., Anal. Ed.*, **10**, 326 (1938).
6. Chemical Service and Equipment Co., 95 Madison Ave., New York, N. Y.
7. Conway, E. J., *Biochem., J.*, **28**, 283 (1934).
8. Eisner, W., *Z. anal. Chem.*, **91**, 172 (1933).
9. Elek, A., Rockefeller Institute for Medical Research, New York, N. Y., private communication.
10. Emich, F., in A. A. Benedetti-Pichler's, "Die Fortschritte der Mikrochemie in den Jahren 1915–1926," F. Deuticke, Leipzig and Vienna, 1927, p. 152.
11. Flatt, R., *Helv. Chim. Acta*, **17**, 1513 (1935).
12. Freudenberger, K., and Weber, E., *Z. angew. Chem.*, **38**, 280 (1925).
13. Friedrich, A., "Die Praxis der quantitative organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, p. 173.
14. Friedrich, A., *Mikrochemie*, **22**, 251 (1937).
15. Fuchs, H. J., *Biochem. Z.*, **170**, 76 (1926).
16. Geilman, W., and Hoeltje, R., *Z. anorg. Chem.*, **152**, 69 (1926).
17. Guzman, J., *Anales soc. españ. física quím.*, **32**, 1129 (1935).
18. Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, **135**, 46 (1923).
19. Hahn, F. L., *Mikrochemie*, *Pregl Festschrift*, 1929, p. 134; and co-workers, *Z. anal. Chem.*, **69**, 417 (1926); *Z. physik. Chem.*, **127**, 1 (1927).
20. Heatley, N. G., *Biochem.*, I, **29**, 626 (1935); *Mikrochemie*, **26**, 147 (1939).
21. Hellmann, J., *Mikrochemie*, **1**, 48 (1923).
22. Johnson, D. L., and Shrewsbury, C. L., *Mikrochemie*, **26**, 143 (1939).
23. Kagan, S. L., *J. allgem. Chem.* (*Russ.*), **5**, (67), 179 (1936).
24. Katow, I. A., *Betriebs Lab.* (*Russ.*), **5**, 1267 (1936).
25. Khouri, J., *Bull. soc. chim. biol.*, **17**, 1077 (1936).
26. Kirk, P. L., *Mikrochemie*, **14**, 1 (1933).
27. Kirkish, F. J., *Chemist-Analyst*, **29**, 68 (1940).
28. Kolthoff, I. M., and Menzel, H., "Die Massanalyse," J. Springer, Berlin, 1931, p. 377.
29. Kolthoff, I. M., and Furman, N. H., "Volumetric Analysis," Vol. II, John Wiley & Sons, New York, 1929, pp. 109–110.
30. Korenman, I. M., *Lab. Prakt. U. S. S. R.*, **12**, No. 6, 25 (1937); *Zavodskaya Lab.*, **5**, 32 (1936).
31. Krause, W., *Chemist-Analyst*, **26**, No. 2, 44 (1937).
32. Krumholz, D., *Mikrochemie*, **25**, 242 (1938).
33. Laskin, I. E., *Mikrochemie*, **8**, 310 (1930).
34. Linderström-Lang, K., and Holter, H., *Hoppe-Seyler's Z. physiol. Chem.*, **201**, 9 (1931); *Compt. rend. trav. lab. Carlsberg*, **19**, No. 4 and 14 (1933).
35. Lindner, J., *Z. anal. Chem.*, **91**, 105 (1933); *Mikrochemie*, *Molisch Festschrift*, 1936, p. 301.
36. Links, R., *Mikrochemie*, **15**, 87 (1934).
37. Ljungdahl, M., *Biochem. Z.*, **96**, 325 (1919).
38. Lochte, H. L., and Hoover, A., *Ind. Eng. Chem., Anal. Ed.*, **5**, 335 (1933).
39. Lunge, G., *Z. angew. Chem.*, **17**, 195, 225, 231, 265 (1904); **18**, 1520 (1905).
40. Machlett, E., and Son, 220 E. 23rd St., New York, N. Y.
41. Mika, J., "Die exakten Methoden der Mikromassanalyse," F. Enke, Stuttgart, 1940.

42. Niederl, J. B., Niederl, V., and Eitingon, M., *Mikrochemie*, **25**, 143 (1938).
43. Phansalkar, S. L., *Chemistry & Industry*, **56**, 720 (1937).
44. Pregl, F., and Roth, H., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, pp. 183–190.
45. Rehberg, P. B., *Biochem. J.*, **19**, 270 (1925).
46. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 148–154.
47. Schilow, E., *Mikrochemie*, **7**, 144 (1929); *Z. anal. Chem.*, **70**, 23, 35 (1927); **72**, 261 (1927).
48. Schwarz, K., *Mikrochemie*, **13**, 1 (1933).
49. Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, **45**, 376 (1921).
50. Sörensen, S. P. L., and co-workers, *Z. anal. Chem.*, **45**, 217 (1906); *Biochem. Z.*, **21**, 168 (1909).
51. Spatz, W., *Chem. Fabrik*, **9**, 70 (1936).
52. Standen, G. W., and Fuller, M. L., *Ind. Eng. Chem., Anal. Ed.*, **6**, 299 (1934).
53. Thimann, K. V., *Chemist-Analyst*, **22**, 21 (1934).
54. Thomis, C. N., *Praktika Akad. Athenon*, **11**, 317 (1936).
55. Thunberg, T., *Skand. Arch. Physiol.*, **72**, 291 (1936).
56. White, E. P., *Ind. Eng. Chem., Anal. Ed.*, **10**, 668 (1938).
57. Widmark, E. M. P., and Oerskov, S. L., *Mikrochemie*, **7**, 293 (1939); *Biochem. Z.*, **201**, 15 (1928).
58. Yagoda, H., *Chemist-Analyst*, **22**, 18 (1934).
59. Zacherl, M. K., and Krainick, H. G., *Mikrochemie*, **11**, 61 (1933).
60. Zakrzewski, Z., and Fuchs, H. J., *Biochem., Z.*, **285**, 390 (1936).

ELEMENTARY ANALYSIS

I. DETERMINATION OF METALS

Principle

Metals in organic compounds are determined by combusting the substance in the presence of dilute sulfuric acid, or by simply burning off the organic constituents and weighing the remaining residue as sulfate (*a*), or metal (*b*).

$$(a) \qquad 2RCOONa \xrightarrow[\Delta]{+H_2SO_4} Na_2SO_4$$

$$(b) \qquad RCOOAg \xrightarrow{\Delta} Ag$$

Apparatus

The Micromuffle (Fig. 14). The micromuffle consists of two hard-glass tubes of Supremax or Pyrex glass 172. One is a horizontal combustion tube 18 to 20 cm. long and at least 8 mm. in inside diameter; the other is a rectangular tube, the vertical arm of which is approximately 15 cm. long and the horizontal arm about 8 cm. long and wide enough to permit the insertion of the horizontal combustion tube. A layer of asbestos paper is placed between the two tubes to provide a

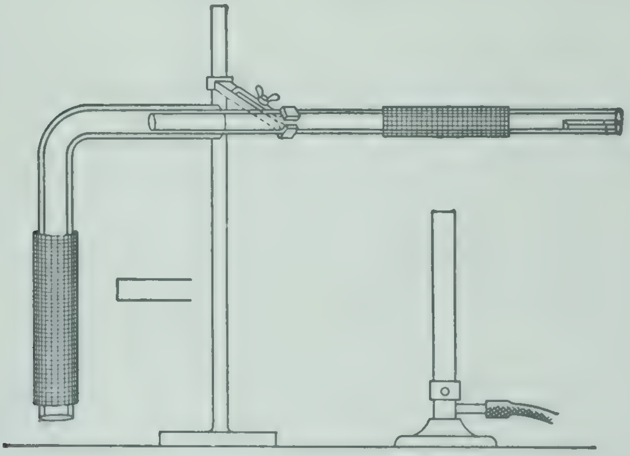


FIG. 14. Micromuffle.

well-fitting connection and to prevent them from cracking when heated. Both tubes may be heated by either Bunsen burners or electrical heating units. Wire gauzes of nichrome wire should be used for the protection of the combustion tube. The vertical tube is heated to supply a hot air current for the combustion and to facilitate the evaporation of volatile pyrolysis products. Electrical heating units offer the advantage of a more efficient and equal distribution of heat during the combustion, thereby preventing the substance from creeping over the boat. Their temperature should be regulated to be between 600 and 700° C.

Platinum Cylinder. To prevent the loss of substance because of its tendency to froth and spatter during the combustion, a platinum cylinder is used as a protective covering for the boat. This cylinder is made of platinum foil 0.04 mm. thick, is 3 cm. long, and has a diameter of 7.5 cm., i.e., a little less than that of the horizontal tube of the micromuffle. A thin platinum wire about 2 cm. long is welded to the cylinder and the end of the wire is bent to form a hook which facilitates the manipulation of the cylinder.

Reagents

Sulfuric Acid (sp. gr.: 1.84). Diluted 1 : 5 with distilled water.
Nitric Acid (sp. gr.: 1.42). Diluted 1 : 1 with distilled water.

Procedure

The amount of substance to be taken depends upon the balance on which it is weighed; thus 3 to 5 mg. is taken if a microanalytical balance is employed or about twice as much if an ordinary analytical balance is used. The zero reading of the balance is determined first, and then the previously cleaned platinum cylinder and boat are weighed against a counterpoise, after which the required amount of substance is placed in the boat and both weighed again. The boat is placed on the glass cover of the microdesiccator and one or two drops of dilute sulfuric acid is added to the sample with a capillary. The boat is inserted in the platinum cylinder and both are placed far enough inside the horizontal combustion tube so that only the hook of the platinum wire protrudes from the opening. Heating is started with a slightly hissing flame approximately 5 cm. from the platinum cylinder and the burner is gradually moved toward the cylinder until it is reached, an operation which requires about 5 minutes. When no more fumes are given off the burner is placed directly under the cylinder and the heating continued with a somewhat stronger flame for ten minutes. Then, with the boat left inside the platinum cylinder, both are removed from the combustion tube with steel or platinum-tipped forceps, held in the outer cone of a non-luminous flame until dark red, placed on the metal block of the microdesiccator, and weighed after five minutes. During these five minutes while boat and cylinder are permitted to cool, the zero reading of the balance should be determined so that any deviation can be corrected for. Should the residue show black particles, thus indicating incomplete combustion, another drop of dilute sulfuric acid is added and the combustion repeated, but the combustion tube is permitted to cool before both platinum cylinder and boat are introduced.

This determination may also be carried out without the platinum cylinder. The procedure remains the same, except that the boat must be approached more slowly with the flame in order to reduce the creeping of the substance to a minimum. Caution must also be exercised in removing the boat from the combustion tube, lest particles which have crept over be scraped off and lost.

Time:

	Minutes
Determination of the zero reading	5
Weighing of the boat	5
Weighing of the sample	5
Combustion	15
Determination of the zero reading	5
Weighing of the residue	5
Total	40

Calculation:

Log of weight of sulfate or residue,*
Plus log of factor,†
Plus negative log of weight of sample;
Antilog (numerus) of total = percentage of metal.

Remarks

This method is suitable for the determination of the following metals in organic compounds: barium, calcium, potassium, and sodium [2] as sulfates; silver (salts), gold (aurates), and platinum (platinates) as metals. The literature [5, 9, 11, 12] also reports successful application of this method to cadmium, cesium, lead, lithium, magnesium, manganese, rubidium,[12] strontium, and zinc, which are determined as sulfates. With lead, dilute nitric acid must be added to prevent possible reduction. By simple combustion in a porcelain boat, possibly with the addition of dilute nitric acid, aluminum (Al_2O_3), chromium (Cr_2O_3), copper (CuO), iron (Fe_2O_3), magnesium (MgO), molybdenum (MoO_3), silicon (SiO_2), and tin (SnO_2) may be determined as oxides. Nickel and cobalt are determined as metals;[13] the combustion, however, is carried out in a stream of hydrogen.[4] A considerable improvement is the use of a platinum cylinder as described first by H. I. Coombs[3] and applied to this method by H. K. Alber.[1] Electric heating has been suggested by C. J. Rodden.[10] An automatic apparatus has been described by A. R. Norton, G. L. Royer, and R. Koegel.[8] Suitably constructed torsion microbalances[6] might be used for routine as well as lecture demonstrations. Residue determinations on samples weighing only a few micrograms have been reported by E. Wiesenberger.[14]

---

* See p. 297.

† Silver, gold, and platinum, as metallic residues, require no factor.

LITERATURE

1. ALBER, H. K., *Mikrochemie*, **18**, 95 (1935).
2. CANESSA, J. C., *Rev. facultad cienc. quím.* (*Univ. nac. La Plata*), **10**, 87 (1936).
3. COOMBS, H. I., *Biochem. J.*, **21**, 404 (1927).
4. FRIEDRICH, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, p. 125.
5. HELLER, K., *Mikrochemie*, **12**, 327, 375 (1933); **14**, 369 (1934).
6. JENDRASSIK, L., and DZIOBECK, L., *Magyar Orvosi Archiv.*, **37**, 415 (1937).
7. NIEDERL, J. B., and SILBERT, E. P., *J. Am. Chem. Soc.*, **51**, 376 (1929).
8. NORTON, A. R., ROYER, G. L., and KOEGEL, R., *Ind. Eng. Chem., Anal. Ed.*, **12**, 121 (1940); *News Ed.*, **17**, 521 (1939).
9. PREGL, F., and ROTH, H., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, pp. 170–171.
10. RODDEN, C. J., *Mikrochemie*, **18**, 97 (1935).
11. ROTH, H., and DAW, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 136–140.
12. ROTH, H., *Mikrochemie*, **21**, 227 (1937).
13. STARY, Z., *Mikrochemie*, **15**, 140 (1934).
14. WIESENBERGER, E., *Mikrochemie*, **10**, 10 (1932).

II. DETERMINATION OF THE NEUTRALIZATION EQUIVALENT

IONIC HYDROGEN, CARBOXYL

Principle

The neutralization equivalent of an acid is the number expressing in grams the quantity of the compound required for the neutralization of 1 liter of normal alkali. For monobasic acids it is identical with the number representing the molecular weight; for polybasic acids it is a submultiple of this number.

$$RCOOH + NaOH \rightarrow RCOONa + H_2O$$

On a microscale the method[6, 7] involves the titration of 4 to 8 mg. of an organic acid with standard 0.01 *N* sodium hydroxide solution while such factors as precaution against the absorption of carbon dioxide, the solubility of the acid, and the dissociation of the sodium salt are observed. To overcome the first, the solution is boiled near the end point; through the addition of ethyl alcohol the second and third are satisfactorily remedied.

Apparatus

Microburets, buret stand, titration flasks, dropping bottle, and steaming apparatus as described under *Standard Solutions* on pp. 51–61 are required.

Reagents

0.01 *N Sodium Hydroxide Solution* (p. 56).

0.01 *N Potassium Biiodate Solution* or 0.01 *N Hydrochloric Acid Solution* (p. 54).

Phenolphthalein Indicator Solution (p. 54).

Neutral Ethyl Alcohol (50%). One hundred milliliters of 95% ethyl alcohol is diluted with 100 ml. of distilled water; 2 to 4 drops of phenolphthalein indicator is added; the solution is boiled for about 30 seconds and then titrated to a slight pink coloration. The neutralization should be repeated from time to time.

Procedure

The substance is weighed in the weighing tube and transferred to a clean and steamed-out, but cool, Erlenmeyer flask of 50-ml. capacity. The substance is dissolved in 5 ml. of 50% neutral ethyl alcohol, warmed if necessary to get the sample in solution, and then titrated with 0.01 *N* sodium hydroxide until the end point is almost reached, as observed when the pink coloration just barely disappears upon shaking. At this point the solution is boiled for about 20 seconds to expel any carbon dioxide absorbed from the air and then it is quickly titrated to the end point, which is reached when the pink coloration is permanent for about one minute. If the end point is passed, 0.01 *N* acid is added in slight excess, the solution boiled again for a few seconds and then titrated back. If the solutions are not exactly 0.01 *N*, their respective factors must be determined as previously described and the necessary corrections applied.

Time:

	Minutes
Weighing the weighing tube with the sample..........	5
Weighing the empty weighing tube.................	5
Titration......................................	15
Total......................................	25

Calculation:

Neutralization Equivalent:

Negative log of ml. 0.01 *N* NaOH used,
Plus log of weight of sample;
Antilog of total = neutralization equivalent, or 1, $\frac{1}{2}$, $\frac{1}{3}$ of molecular weight.

Percentage of Carboxyl:

Log of ml. 0.01 *N* NaOH used,
Plus log of factor (65331),
Plus negative log of weight of sample;
Antilog of total = percentage of carboxyl.

Remarks

The foregoing method is generally applicable to non-amphoteric acids, some lactones and anhydrides, some polynitrophenols, and acidic esters. No reports are given in the literature concerning a satisfactory application of this method to neutral esters. Phenolphthalein is used as indicator, although any other indicator having a similar *p*H range can be substituted for it. Application of this method to the analysis of binary mixtures of benzene carboxylic acids was proved feasible by

W. R. Kirner.[5] Conductometric titrations of organic acids on a microscale have been performed by M. Furter and H. Gubser.[2]

The above method can also be used for the titration of amino acids,[1, 8, 9] as shown by W. Grassmann and W. Heyde.[3]

The weighed amino acid is placed in a glass-stoppered 1-ml. volumetric flask and is dissolved in a minimum of water. By means of calibrated capillary pipets this solution is transferred to a steamed-out titration flask. Two drops of a 0.1% alcoholic indicator solution of thymolphthalein is introduced, and 0.01 *N* alcoholic sodium hydroxide solution is added from the microburet until a distinct blue coloration is obtained.

An amount of absolute alcohol equivalent to 9 times the volume of the original solution of the amino acid is added with a 5-ml. pipet having 0.1-ml. graduations. Upon the addition of the absolute alcohol the blue coloration disappears, and more of the standard alcoholic sodium hydroxide solution is added until the blue coloration is again obtained.

After the determination, a blank titration with exactly the same volume of absolute alcohol as was taken for the dilution of the amino acid is carried out and the amount of 0.01 *N* alcoholic sodium hydroxide solution used is deducted from the volume of alkali required for the neutralization of the amino acid solution.

This method gives satisfactory results provided that the amino acid solution is not excessively diluted. For a 0.01 *N* dilution, 10 times the amount of alcohol has to be added; with greater dilutions the concentration of alcohol may be increased to 80%, which, according to L. J. Harries,[4] is still sufficient to retard the hydrolysis of the sodium salt of the amino acid formed in the titration.

LITERATURE

1. Block, R. J., "The Determination of Amino Acids," Burgess Publishing Co., Minneapolis, Minn., 1938.
2. Furter, M., and Gubser, H., *Helv. Chim. Acta*, **21**, 1725 (1938).
3. Grassmann, W., and Heyde, W., *Z. physiol. Chem.*, **183**, 32 (1929).
4. Harries, L. J., *Proc. Royal Soc.*, (B) **95**, 500 (1923).
5. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **17**, 521 (1939).
6. Niederl, J. B., Niederl, V., and Eitingon, M., *Mikrochemie*, **25**, 143 (1938).
7. Roth, H., and Daw, E. B., "Quantitative Microanalysis of Fritz Pregl,' P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 148–154.
8. Waldschmidt-Leitz, E., *Z. physiol. Chem.*, **132**, 194 (1924).
9. Willstätter, R., and Waldschmidt-Leitz, E., *Ber.*, **54**, 2988 (1921).

III. VOLUMETRIC DETERMINATION OF AMINOID NITROGEN

KJELDAHL METHOD

Principle

The organic substance containing aminoid nitrogen is decomposed in the presence of concentrated sulfuric acid and suitable catalyzers to yield ammonium sulfate quantitatively.[18] After liberation with strong alkali, the ammonia is distilled quantitatively into an excess of 0.01 N acid. The excess acid is titrated back with 0.01 N alkali. The reactions involved are as follows (using urea as an example):

(*a*) $H_2NCONH_2 \xrightarrow[H_2SO_4]{\Delta} (NH_4)_2SO_4$ (digestion)

(*b*) $(NH_4)_2SO_4 + 2NaOH = 2NH_3 + Na_2SO_4$ (distillation)

(*c*) $NH_3 + KH(IO_3)_2 = KIO_3 + NH_4IO_3$ (titration)

Apparatus

Distillation Apparatus [28] (Fig. 15). A 1-liter round-bottomed Pyrex flask, two-thirds filled with distilled water, serves as a steam generator. Pieces of silicon carbide, or porous tile, or a trace of zinc dust is placed in the flask to insure even boiling. The steam generator is connected to the side arm of the cylindrical steam trap, which is provided with a drain arrangement at the bottom. A glass tube, bent at right angles at the top of this steam trap, forms a glass-to-glass connection of this part of the apparatus with the sealed-in steam delivery tube of the distilling flask. The steam delivery tube extends to the bottom of the distilling chamber and has an ascending side arm at the upper part, to which a small funnel of about 10-ml. capacity is attached with a short piece of rubber tubing carrying a pinch clamp. This funnel serves for the introduction of the digestion material into the distilling flask. The distilling flask proper is 30 to 35 cm. long and consists of a distilling or reaction chamber which widens at the top to a foam bulb; a second foam bulb, provided with a curved outlet tube, is sealed to the first. The distilling chamber is surrounded by a glass jacket, which is evacuated, thus eliminating the necessity of heating this part of the apparatus. The distilling flask is held in an oblique position, so that the introduction tube leading into the distilling chamber is vertical. To lessen the danger of contamination of the distillate by the rubber connection, this outlet tube is attached directly to the short vertical arm of the silver tube condenser, which has an outside diameter of 7 mm. To make the glass-to-silver tube connection as tight as possible, the outlet tube should have the same outside diameter as the silver tube condenser. This part of the silver tube rises vertically for about 4 cm. and then ascends obliquely for a length of about 10 cm. and then is bent at right angles to descend vertically. A water-cooling jacket with a suitable water-trap funnel below, to prevent water from dripping into the Erlenmeyer flask, surrounds the lower part of the silver tube, which is about 35 cm. long. The end of the tube extends approximately 10 cm. beyond the cooling jacket and dips into the Erlenmeyer flask below.

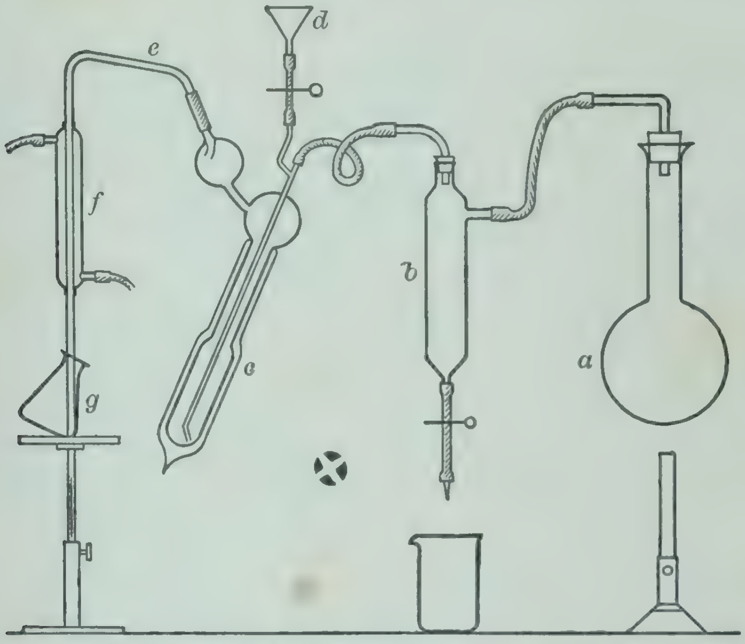


Fig. 15. Distillation Apparatus.[28] *a*, Steam Generator; *b*, Steam Trap; *c*, Distilling Flask; *d*, Introduction Funnel; *e*, Silver Tube Condenser; *f*, Condenser Jacket; *g*, Erlenmeyer Flask.

Digestion Oven (Fig. 16). The digestion oven consists of a metal stand having an asbestos plate with six openings, covered with small round pieces of wire gauze. Centered below the openings and attached to the gas pipe of the stand are six microburners. The fume duct is approximately 42 cm. long and 4 cm. in diameter and is supported by two arms extending upward from the stand. It is connected to a good water suction pump which effectively removes the decomposition vapors generated during the digestion. Electrically heated digestion ovens are also known.

Digestion Flask. Made of Pyrex glass 1 mm. thick, the digestion flask is about 16 cm. long with an outside diameter of 1.5 cm. at the neck and has a bulb-shaped bottom of about 15-ml. capacity for the retention of the reaction material.

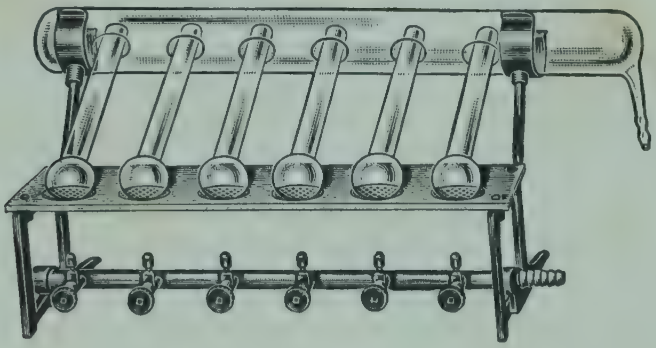


FIG. 16. Digestion Oven.

Titration Equipment. Titration equipment comprises microburets, buret stand, dropping bottle, titration flasks, and a steaming funnel and is described under " Standard Solutions " on p. 51.

Reagents

Concentrated Sulfuric Acid (sp. gr.: 1.84).

Catalyzers. A powdered, nitrogen-free mixture of 1 part potassium sulfate and 3 parts of copper sulfate;[30, 32] powdered selenium;[14, 19] mercuric acetate, or mercuric sulfate.[26, 36]

Perhydrol. Nitrogen-free hydrogen peroxide, 30%.

Sodium Hydroxide Solution (30%). This solution is prepared by dissolving 240 grams of C.P. sodium hydroxide in 560 ml. of distilled water.

0.01 *N Sodium Hydroxide Solution* (p. 56).

0.01 *N Acid Solution* (potassium biiodate[25] or hydrochloric acid) (p. 54).

Methyl Red Indicator Solution (p. 54). This indicator solution is stored in a standard indicator bottle of about 50-ml. capacity, having a ground-glass stopper which is drawn out to a glass rod about 1 mm. thick so that no more than a trace of the solution can be taken at one time.

Procedure

Solids. The substance is weighed in the weighing tube and transferred to a clean, dry Kjeldahl digestion flask by holding the digestion flask in a horizontal position while introducing the weighing tube containing the sample. The digestion flask is then brought to a vertical position while the weighing tube is held as far as possible within the flask. By this manipulation most of the substance will fall directly into the bulb of the digestion flask and spilling of material on the wall of the neck of the flask will be avoided. The weighing tube is then withdrawn and weighed again.

Liquids. The liquid is weighed in a sealed capillary and placed in the digestion flask. After addition of the sulfuric acid the capillary is broken with a clean glass rod, which is rinsed with a few drops of sulfuric acid. For sticky syrups and heavy oils flat-bottomed weighing cups or bottles can be used.

Solutions. Solutions of liquids or solids, or biological material containing not more than 1 mg. of nitrogen per milliliter, are introduced into the digestion flask by means of a precision pipet of the weighing or wash-out type, which is provided with a rubber tubing ending in a mouthpiece of glass.[30, 32] A small cotton wad is placed in this rubber tubing near the mouthpiece to prevent saliva from entering the pipet when its contents are being blown out. The pipet is rinsed once with the solution to be analyzed, then filled by drawing up the liquid to a few millimeters beyond the calibration mark. The tip of the pipet is wiped clean with a small cotton wad or filter paper, and, by still holding the pipet in a horizontal position and touching the tip with filter paper, the excess liquid is withdrawn. If the calibration is missed when withdrawing the excess, and provided that the level of the liquid has not receded into the bulb-shaped part, the pipet can readily be refilled by dipping it again into the solution. The measured portion is transferred to the digestion flask by holding the pipet as far inside the digestion flask as possible, without touching the side wall, however, and blowing out the contents. The tip of the pipet is rinsed with a few drops of distilled water from a wash bottle before it is withdrawn from the digestion flask. To complete the quantitative transfer of the solution the pipet is rinsed twice with distilled water and the washings transferred to the digestion flask in the same manner. Should bubbles form in the pipet, owing to improper manipulation in aspirating or blowing out the solution, the pipet is cleaned by rinsing it with ethyl alcohol and drying it on a suction pump.

Digestion. A knife point of selenium or potassium sulfate and copper sulfate mixture is added to the sample in the digestion flask, and then 1 ml. of concentrated sulfuric acid is introduced which may be withdrawn from a macroburet set up for this purpose. The digestion flask is placed on the digestion oven and at first slowly, then vigorously, boiled until the contents have become perfectly clear and straw yellow or light green in color. Depending on the type of substance to be analyzed, fifteen to twenty minutes of boiling is generally sufficient; but if the digestion is still incomplete after this time, 2 to 3 drops of perhydrol is added to the digestion material and the heating continued for five to ten minutes. The last step may be repeated if the substance is extremely difficult to decompose.

Distillation. Approximately 7 ml. 0.01 *N* acid, accurately measured, is run from the buret into a 50-ml. steamed-out Erlenmeyer flask of Pyrex glass or quartz, to which a trace of methyl red indicator has been added. (Should the first distillation indicate that 7 ml. 0.01 *N* acid is not enough or much in excess, depending on the nitrogen content of the substance, correspondingly more or less 0.01 *N* acid is used for the following distillation; however, an excess of 1 to 2 ml. 0.01 *N* acid must be maintained.) Next, the cooled material in the digestion flask is diluted with 2 ml. of distilled water and cooled again under the tap.

With the water in the steam-generating flask boiling moderately, and the pinch clamp at the bottom of the steam trap open to provide an outlet for the steam, the contents of the digestion flask are quantitatively transferred to the distilling chamber of the apparatus by pouring the digestion material and two 2-ml. washings of distilled water into the small funnel on top of the distilling flask. This operation is aided by rinsing the mouth of the digestion flask with a few drops of distilled water from a wash bottle, or by making the outside of the rim of the digestion flask water-repellent through the application of a trace of stopcock grease. Then the Erlenmeyer flask containing the 0.01 *N* acid is placed under the silver tube condenser and raised until the tip of the tube dips well below the liquid. With the aid of a pipet 7 ml. of 30% sodium hydroxide is poured through the introduction funnel into the distilling chamber, which is sufficient to make the reaction material decidedly basic.

To begin with the distillation itself, the pinch clamp on the funnel and then the pinch clamp at the bottom of the steam trap are closed, forcing the steam to pass from the steam generator to the distilling flask. The duration of the distillation is exactly three minutes, counting from the time when the condensate enters the outlet tube of the second foam bulb of the distilling flask. After this time the Erlenmeyer flask is lowered until the tip of the silver tube condenser is about

1 cm. above the liquid, and the distillation continued for another minute. Before the Erlenmeyer flask is removed from the apparatus, the tip of the silver tube condenser is rinsed with a few drops of distilled water. Should the pink coloration of the liquid in the Erlenmeyer flask have become too faint, another trace of methyl red indicator may be added; but if the solution has turned yellow during the distillation, indicating the change from acid to alkali, the analysis must be considered spoiled because of the presence of insufficient 0.01 *N* acid to absorb quantitatively the ammonia distilling over. After the distillation the residue in the distilling chamber is automatically transferred to the steam trap by creating sufficient vacuum through the removal of the flame from under the steam generating flask. No further cleaning of the distilling flask is necessary, and the apparatus is ready for the next determination. The residue collected in the steam trap is drained off by opening the pinch clamp at the bottom.

Before starting a series of analyses, especially if the apparatus has not been in use for some time, it is necessary to steam out the entire system for about thirty minutes by closing first the pinch clamp at the bottom of the steam trap and five minutes later also the pinch clamp under the introduction funnel. An Erlenmeyer flask is placed under the silver tube condenser to receive the water distilling over. It is also advisable to test the apparatus as well as the purity of the reagents by a blank distillation. A properly set up and maintained apparatus and correctly prepared reagents should give no blank values.

Titration. The solution in the Erlenmeyer flask is boiled for about 20 seconds to expel carbon dioxide absorbed from the air and titrated to the end point with 0.01 *N* sodium hydroxide solution; the end point is reached when a canary-yellow coloration is obtained. If the 0.01 *N* solutions are not exactly 0.01 *N*, the respective factors must be determined as described on pp. 55-56 and the necessary corrections applied.

Time:

	Minutes
Weighing or pipetting of the sample	15
Digestion	20
Distillation	10
Titration	10
Total	55

Calculation:

Log of ml. of 0.01 *N* acid neutralized,
Plus log of factor (14638),
Plus negative log of weight of substance;
Antilog of total = percentage of nitrogen.

Remarks

The volumetric determination of nitrogen by the Kjeldahl method [18, 28, 29, 30, 32, 35] is generally applicable to substances containing aminoid nitrogen, which yield ammonia quantitatively during the digestion process. Thus the applicability of this procedure, without the introduction of reducing agents, is limited to amines, amino acids, ureas, thio ureas, and acid amides, provided that their structure is not too complex. Proteins yielding decomposition as well as hydrolysis products of a similar generic structure can also be determined by this method (factor: 6.25), as the wide application of this method in the analysis of biological or physiological fluids (urine, blood, etc.) indicates.[1, 11, 39] It is suggested, however, that a test analysis of a known sample of the same or related structure be made first, or the determination be checked by a gasometric nitrogen (Dumas) determination.

Successful attempts to widen the applicability of this method by the introduction of reducing agents, such as reducing sugars (glucose) and hydriodic acid, have been reported by A. Elek and H. Sobotka,[8] R. A. Harte,[13] and A. Friedrich.[11] Their experimental findings illustrate the successful application of this method, with the appropriate modifications, to nitroso (β-nitrosonaphthol) and nitro compounds, aromatic amines, hydroxyl amines (oximes), hydrazines (substituted hydrazones, osazones), and some azo and diazo compounds.

For the reduction process with glucose the following quantities of reagents are employed: 3- to 10-mg. sample, 100 mg. glucose, 100 mg. potassium sulfate, 2 to 3 mg. copper sulfate or mercury or mercury acetate, 3 ml. concentrated sulfuric acid.

With hydriodic acid: 3- to 5-mg. sample, 1 ml. hydriodic acid (sp. gr.: 1.7), 2 to 3 mg. of red phosphorus and mercuric acetate, 100 mg. potassium sulfate.

The micro Kjeldahl method has been repeatedly discussed and its various phases scrutinized.[2, 3, 21, 27, 34, 41] The discussion included: catalyzers, the sulfates of potassium,[30, 32] copper,[30, 32] mercury and mercuric oxide,[26, 37] iron,[28] magnesium,[38] and selenium;[14, 19] mixed indicators (methyl red and methylene blue, etc.;[15] one-piece apparatus,[4, 5, 10, 16, 17] of which the apparatus of P. L. Kirk [17] has found the widest application.

This apparatus (Fig. 17) consists of a distillation flask *a* of 35-ml. capacity. This flask is inclosed in a glass jacket *b* which serves as a steam generator. A Y-shaped tube is sealed to the inner wall above the steam generator, one arm permitting the passage of steam from the steam generator and the other the introduction of the reaction material through funnel *c*. Above the flask is a trap *d* and head leading to a condenser *e*, which drains through a vertical delivery tube into the receiver. An overflow and by-pass carry the water from the condenser water to the drain or to the steam generator, which is equipped with a drain tube. The total height of the apparatus is 35 cm. The steam generator jacket is 4.2 cm. in diameter and 16 cm. in height, the bulb being 6 cm. in diameter. The internal effective portion of the condenser is 1.8 cm. in diameter and 5.5 cm. in length.

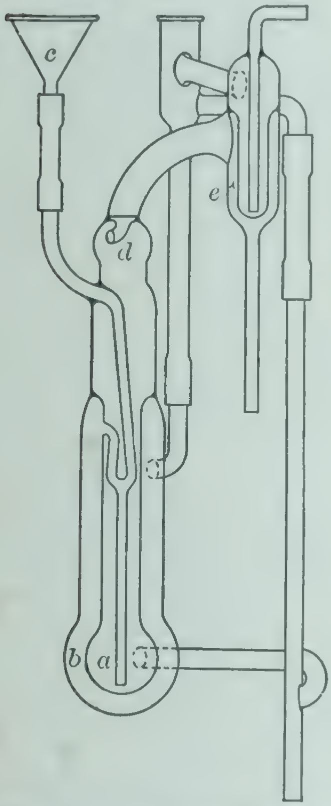


FIG. 17. One-Piece Distillation Apparatus.[17] Explanation in Text.

Around the steam generator may be wrapped an insulating asbestos jacket to prevent sudden cooling from drafts. A few pieces of porous tile or silicon carbide are placed in the generator to prevent irregular boiling. A moderate flame should be used for the initial heating, and a strong flame as soon as the water begins to boil.

The use of the micro Kjeldahl method without a microanalytical balance[14, 25] and the utilization of boric acid[40] in the titration for series determinations[7, 20, 37] have been described, and adaptation to a semimicro procedure (20–60 mg.)[31] as well as for ultramicroanalysis (micrograms) have been reported.[17, 23] Application of the micro Kjeldahl method to nitrogen determinations in coal[3] and dyestuffs[33] as well as simultaneous determination of arsenic and chlorine[5] have proved feasible.

An iodometric Kjeldahl method has been described by I. Bang[1] involving the iodometric determination of the residual free standard acid. This procedure is particularly applicable to substances with a low nitrogen content; the use of perhydrol and indicator, however, must be avoided. After distillation, the 0.01 N or 0.005 N acid is boiled for a few seconds to expel the carbon dioxide and then allowed to cool. Two to three milliliters of 5% potassium iodide and 1 ml. of 4% potassium iodate solution are added. After five minutes, during which time the solution is shaken repeatedly, the iodine is titrated with 0.01 N or 0.005 N sodium thiosulfate solution. A critical discussion of the iodometric nitrogen determination has been given by S. Morgulis and A. F. Friedman.[22]

Of the other methods for the determination of aminoid nitrogen, the manometric method of D. D. Van Slyke,[39] which also has found extensive clinical use,[6, 24] is the most important.

LITERATURE

1. Bang, I., "Methoden zur Mikrobestimmung einiger Blutbestandteile," J. F. Bergmann, Wiesbaden, 1916.
2. Bartosiewicz, S. Z., *Biochem. Z.*, **289**, 55 (1936).
3. Beet, A. E., and Belcher, R., *Mikrochemie*, **24**, 145 (1938).
4. Brant, J. H., and Sievers, D. C., *Ind. Eng. Chem., Anal. Ed.*, **13**, 133 (1941).
5. Das Gupta, H. N., *J. Indian Chem. Soc.*, **14**, 358 (1937).
6. Dumazert, C., *Bull. soc. chim.*, (5) **6**, 42 (1939); *Bull. soc. chim. biol.*, **20**, 1405 (1938).
7. Eisner, A., and Wagner, E. C., *Ind. Eng. Chem., Anal. Ed.*, **6**, 473 (1934).
8. Elek, A., and Sobotka, H., *J. Am. Chem. Soc.*, **48**, 501 (1926).
9. Ferguson, G. E., and Scheflan, L., *Ind. Eng. Chem., Anal. Ed.*, **12**, 553 (1940).
10. Fife, J. M., *Ind. Eng. Chem., Anal. Ed.*, **8**, 316 (1936).
11. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, pp. 202–204; *Z. physiol. Chem.*, **216**, 68 (1933).
12. Gibbs, G. E., and Kirk, P. L., *Mikrochemie*, **16**, 25 (1934).
13. Harte, R. A., *Ind. Eng. Chem., Anal. Ed.*, **7**, 432 (1935).
14. Hartley, O., *Ind. Eng. Chem., Anal. Ed.*, **4**, 249 (1934).
15. Johnson, A. H., and Green, J. R., *Ind. Eng. Chem., Anal. Ed.*, **2**, 2 (1930).
16. Kemmerer, G., and Hallett, L. T., *Ind. Eng. Chem.*, **19**, 1925 (1927).
17. Kirk, P. L., *Mikrochemie*, **16**, 13 (1934); *Ind. Eng. Chem., Anal. Ed.*, **8**, 223 (1936).
18. Kjeldahl, J., *Z. anal. Chem.*, **22**, 366 (1883).
19. Lauro, M. F., *Ind. Eng. Chem., Anal. Ed.*, **3**, 401 (1931).
20. Meeker, E. W., and Wagner, E. C., *Ind. Eng. Chem., Anal. Ed.*, **5**, 396 (1933).
21. Miller, H. S., *Ind. Eng. Chem., Anal. Ed.*, **8**, 50 (1936).
22. Morgulis, S., and Friedman, A. F., *Bull. soc. chim. biol.*, **18**, 1074 (1937).
23. Needham, J., and Boell, E. J., *Biochem. J.*, **33**, 149 (1939).
24. Nicloux, M., *Compt. rend. soc. biol.*, **129**, 1171 (1938).
25. Niederl, J. B., Niederl, V., and Eitingon, M., *Mikrochemie*, **25**, 143 (1938)
26. Osborn, R. A., and co-workers, *J. Assoc. Official Agr. Chem.*, **18**, 604 (1935); **16**, 107 (1933).
27. Parnas, J. K., *Z. anal. Chem.*, **114**, 261 (1938).
28. Parnas, J. K., and Wagner, R., *Biochem. Z.*, **125**, 253 (1931).
29. Pilch, F., *Monatsh.*, **32**, 21 (1911).
30. Pregl, F., and Roth, H., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, pp. 105–114.
31. Redeman, C. E., *Ind. Eng. Chem., Anal. Ed.*, **11**, 635 (1939).
32. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son and Co., Philadelphia, Pa., 1937, pp. 86–89.
33. Schaposchnikow, W. G., *Mem. Inst. Chem. Techn. Acad. Sci. Ukraine*, **5**, 59 (1937).
34. Schulek, E., and Vastagh, G., *Z. anal. Chem.*, **92**, 352 (1933).

35. Scott, J. E., and West, E. S., *Ind. Eng. Chem., Anal. Ed.*, **9**, 50 (1937).
36. Screenivasan, A., and Sadavisan, V., *Ind. Eng. Chem., Anal. Ed.*, **11**, 314 (1939).
37. Sobel, A., Yuska, H., and Cohen, J., *J. Biol. Chem.*, **118**, 443 (1937).
38. Stubblefield, F. M., and Deturk, E. E., *Ind. Eng. Chem., Anal. Ed.*, **12**, 396 (1940).
39. Van Slyke, D. D., and co-workers, *J. Biol. Chem.*, **102**, 489, 651 (1933); **71**, 235 (1927).
40. Wagner, E. C., *Ind. Eng. Chem., Anal. Ed.*, **12**, 771 (1940).
41. Zakrzewski, Z., and Fuchs, H. J., *Biochem. Z.*, **285**, 390 (1936).

IV. GASOMETRIC DETERMINATION OF NITROGEN

DUMAS METHOD

Principle

The gasometric determination of nitrogen by the Dumas method is applicable to any organic compound containing nitrogen in any form, such as amino, nitroso, nitro, azo, cyano, alkyl nitrites or nitrates, or heterocyclic nitrogen compounds. The substance is combusted in a closed system in an atmosphere of carbon dioxide; copper oxide is used as oxidizing agent, and metallic copper for the reduction of oxides of nitrogen to elementary nitrogen. To control the error due to the residual air in the carbon dioxide, which is determined separately in a blank determination, the combustion is carried out with a measured volume of carbon dioxide. The liberated nitrogen is collected quantitatively over 50% potassium hydroxide solution in a nitrometer.

$$\text{Organic nitrogen compound} \xrightarrow[\text{CuO}]{\Delta(\text{CO}_2\ \text{atm.})} x\text{CO}_2 + x\text{H}_2\text{O} + \text{N}_2$$

Apparatus

Kipp Generators (Fig. 18, I and II). The Kipp gas generators I and II have a generating-chamber capacity of 2 liters and are connected, as shown in the illustration, by a glass tube carrying a three-way stopcock *A*, one arm of which is bent upward to extend above the marble in the generating chamber of Kipp I, while the other arm is connected to a glass tube leading into the top bulb of Kipp II. Another glass tube extends to the base of Kipp II and is bent at a right angle above the rubber stopper of the top bulb. The generating chamber of Kipp II has the same outlet arrangement as Kipp I, except that the three-way stopcock *B* is connected to the gasometer. Stopcock *A* serves for the deaeration of the generating chamber of Kipp I as well as for the evacuation of the top bulb of Kipp II. Stopcock *B*, aside from being used to deaerate the generating chamber of Kipp II, is also a safety stopcock to shut off the carbon dioxide in case of breakage of the glass tubing leading to the gasometer. Stopcocks *A* and *B* should have a minimum bore of 2 mm. to permit the ready flow of the carbon dioxide from one Kipp generator to the other.

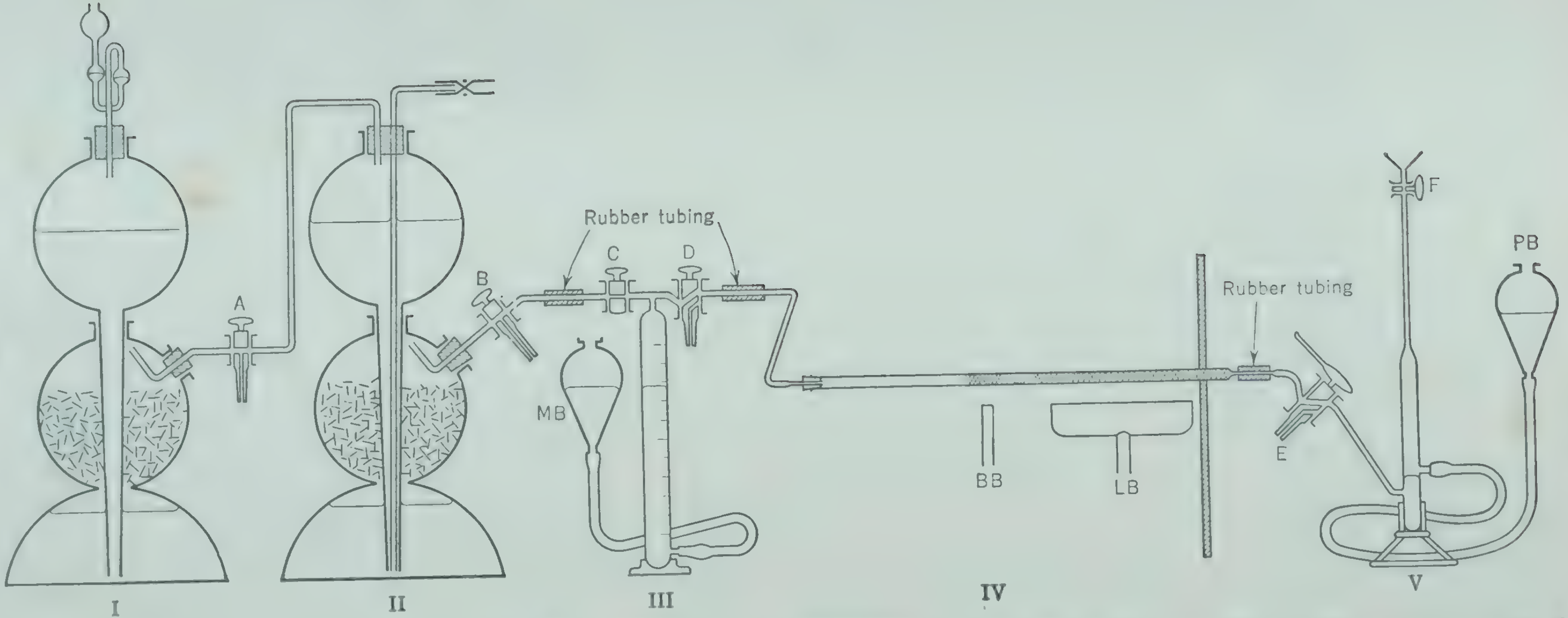

Rubber tubing
C
D
Rubber tubing
A
B
MB
BB
LB
E
F
PB
I
II
III
IV
V

Fig. 18. Dumas Nitrogen Apparatus. I and II, Kipp Generators; III, Gasometer; IV, Combustion Tube; V, Nitrometer. *A, B, C, D, E, F*, Stopcocks; *MB*, Mercury Leveling Bulb; *PB*, Potassium Hydroxide Leveling Bulb; *BB*, Bunsen Burner; *LB*, Long Burner.

Preparation of the Kipp Generators. Because it is necessary to carry out the preparation of the Kipp generators within as short a time as possible, to prevent the prepared marble from absorbing air again, all glass tubes used for the various connections are previously cut to the right length, their ends are glazed, and the tubes bent to fit properly. Well-fitting rubber stoppers for the openings of the Kipp gas generators are also prepared and the glass tubes carrying the stopcocks inserted. With these preparations completed, Kipp generator I is put together, the ground-glass joint being well greased to form a perfect connection. Short glass rods, bent in V-form, are placed around the constriction between the middle and lower bulbs to prevent smaller pieces of marble from falling through. The generating chamber is filled to three-fourths of its capacity with marble, about 2 kg., and the side opening closed with a very tight-fitting rubber stopper carrying stopcock A, which is wired to this opening to withstand the pressure of the carbon dioxide. About 4 liters of hydrochloric acid is poured in, filling the bottom bulb up to the marble and the top bulb to one-half. To expel the air from the generating chamber carbon dioxide is generated repeatedly, but not too violently, by opening and closing stopcock A, thus bringing the hydrochloric acid in contact with the marble.

Kipp generator II, filled in the same manner, is then connected to the first, as shown in the illustration. Kipp I is closed at the top with a rubber stopper carrying a short-stem funnel tube with a double-loop trap containing mercury. The top bulb of Kipp II is closed by a two-hole rubber stopper through which extend two glass tubes; they are suitably bent as shown in Fig. 18. One glass tube extends to the base of the Kipp generator, and the other leads to the generating chamber of Kipp I. · The former glass tube facilitates the convenient renewal of the hydrochloric acid, whenever necessary, without the necessity of disturbing the gas-tight connections of the apparatus. The outlet of this tube may either be sealed off or closed with a strong screw clamp. In order to retain a certain flexibility of the apparatus, thereby avoiding strain and possible breakage of the glass tubings, the various connections are not made glass-to-glass, but the ends of the glass tubings which are to be joined remain about 1 cm. apart, with the rubber tubings connecting them covering 2 to 3 cm. each of the two sections. To make all the rubber stoppers, rubber tubings, and the ground-glass joint between the top bulb and the generating chamber of the Kipp generator gas-tight, they are painted with Glyptal (No. 1201 red) or sealed with Picein. But considering that a properly set up apparatus will give an uninterrupted supply of very pure carbon dioxide for more than a thousand analyses, it is well worth the effort expended on the initial set-up.

With the gas-tight Kipp generators connected as shown in Fig. 18, both generating chambers should again be deaerated as previously described. To reduce the air content of the hydrochloric acid to a minimum, the top bulb of Kipp II is evacuated and the air replaced by carbon dioxide which is withdrawn from the generating chamber of Kipp I. This evacuation is carried out by attaching stopcock *A* by means of rubber tubing to a water-suction pump and turning the plug of the stopcock in such a way that Kipp I is shut off and the air withdrawn from the top bulb of Kipp II. Care must be exercised so that the hydrochloric acid in the bottom bulb does not recede below the wide tube of the Kipp generator. Stopcock *A* is then turned so that the carbon dioxide from the generating chamber of Kipp I fills the evacuated top bulb of Kipp II. By repeating this procedure of deaeration and evacuation several times in succession and again 24 hours later, the carbon dioxide supplied by Kipp II should be of sufficient purity to give satisfactory microbubbles. The purity of the carbon dioxide of a newly set up apparatus is tested by a blank determination as described on p. 94. Should the blank volume exceed the previously stated limits, then the process of deaeration and evacuation is repeated.

Microbubbles. Gas bubbles showing certain characteristics in speed and appearance when rising in the nitrometer are defined as microbubbles. Their characteristics are the following: They should require at least twenty seconds to rise from the surface of the mercury to the top of the nitrometer, be nearly absorbed by the 50% potassium hydroxide solution in the lower, wide part of the nitrometer, overtake each other in the stem, and ascend in a closely packed column. (With the set-up of the Kipp generators as described above, microbubbles showing these characteristics should be readily obtained.) According to F. Pregl the diameter of a microbubble should appear to measure 0.2 of a division of the nitrometer.[43, 44, 47]

Gasometer (Fig. 19). In order to be able to measure the volume of carbon dioxide used for each analysis, a graduated gasometer of about 100-ml. capacity is inserted between Kipp II and the combustion tube. It rests on a grooved wooden block and is held in place by a clamp attached to a metal stand. The gasometer is made of thick-walled glass tubing, has a diameter of about 3 cm., and measures 30 cm. from the bottom to the capillary constriction leading to the T-shaped double stopcock arrangement. About 2.5 cm. from the bottom is an outlet which is connected to a mercury leveling bulb of about 400-ml. capacity by means of a securely fastened rubber tubing. To obtain the pressure necessary for the second phase of the combustion, the leveling bulb is placed in an open ring clamp, which is attached to the metal stand supporting the gasometer. The graduations reading from 0 to 100 ml., begin approximately 9 cm. from the bottom and are subdivided into divisions of 5 ml. The T-shaped arm of the gasometer carries two stopcocks about 5 cm. apart; a glass tubing of 8- to 10-mm. outside diameter and 2.5-mm. inside diameter extends 5 cm. beyond each stopcock. Stopcock *C*, having a plug with a one-way horizontal bore, intersects the glass tubing leading to Kipp II. The plug of the two-way stopcock *D* has an oblique bore which controls the connection leading to the combustion tube, and a downward outlet leading from the gasometer to the outside. This arrangement facilitates the regulation of the gas volume in the gasometer but forestalls the possibility of ruining an analysis by inadvertently turning the stopcock in the wrong direction. The gasometer is connected to the combustion train by a glass tube bent as shown in Fig. 18. The end facing the combustion tube is drawn out to a capillary and provided with a good rubber stopper which fits tightly into the mouth of the combustion tube.

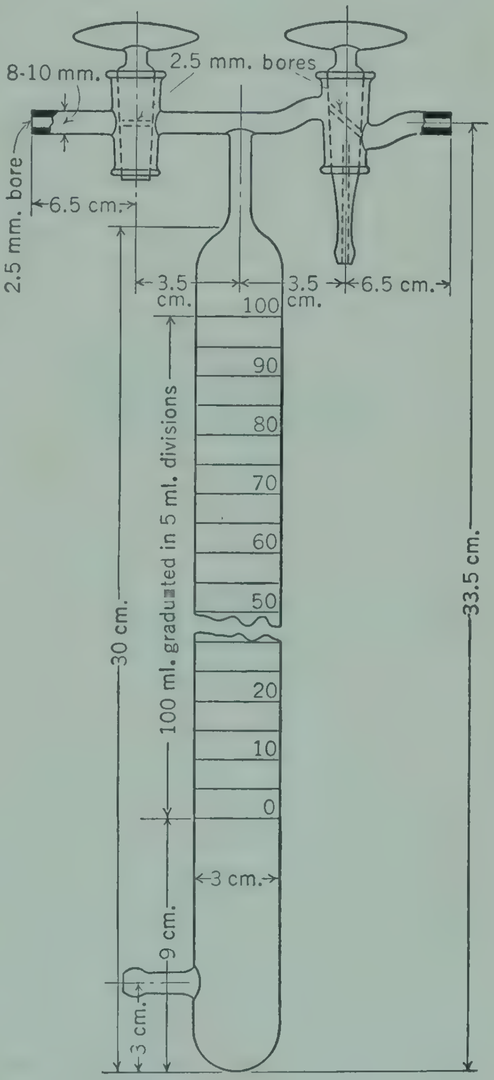


Fig. 19. Gasometer.

Combustion Tube (Fig. 18, IV). The combustion tube is of hard glass (Pyrex 172) or quartz, the latter being preferred when substances have to be combusted at temperatures above 700° C. It is 52 to 54 cm. long and has an inside diameter of 8 mm. (±0.5 mm.). The part facing the nitrometer ends in a capillary of 3-cm. length, 2-mm. inside and 3.25-mm. (±0.25 mm.) outside diameter. The filling of the combustion tube consists of the *permanent* and the *temporary* filling. The permanent filling lasts for about 100 analyses, or until the copper oxide shows evidence of becoming reduced; the temporary filling is renewed for every determination. Before being filled the combustion tube is cleaned by immersion for several hours in cleaning solution, kept for this purpose in a glass eudiometer tube about 50 cm. long and 2 to 3 cm. in inside diameter, then by exhaustive washing with tap water, distilled water and finally rinsing with ethyl alcohol. It is dried on the water suction pump, but its mouth is closed with a dust filter to keep the interior free from particles of dust. When clean and dry, the combustion tube is filled as follows: Pre-ignited asbestos is placed against the capillary constriction with the aid of a glass tube or wooden rod, and compressed to form a fairly compact plug 3 to 4 mm. thick. Coarse copper oxide, pre-ignited at 700° C., or higher, is filled in to a length of 17 cm., the tube being tapped repeatedly during the process of filling so that the copper oxide forms a compact layer. Another asbestos plug 2 to 3 mm. thick is inserted, but not compressed too tight, and is followed by a 4-cm. layer of metallic copper wool or copper wire broken up into lengths of 2 to 3 mm. Copper wool is usually preferred, because it provides a greater surface contact. A third asbestos plug, 3 to 4 mm. in length, concludes the permanent filling, which has a total length of 21 to 22 cm. The combustion tube is placed on the metal stand in such a way that the copper oxide filling facing the nitrometer extends 5 cm. beyond the stand, and the rest of the permanent filling, including the metallic copper layer, is completely within the range of the long burner or electric furnace, which should have an effective heating area 16 to 17 cm. long. After the permanent filling has been heated for about one hour in a slow stream of carbon dioxide, the combustion tube is ready for use.

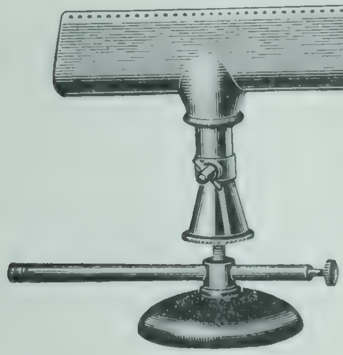


FIG. 20. Long Burner.

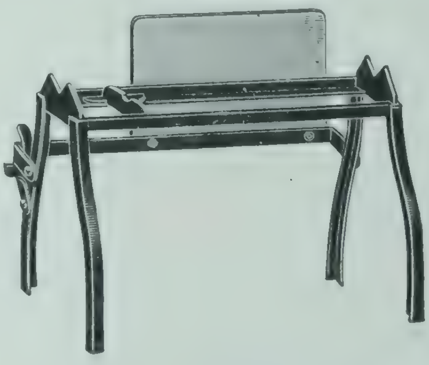


FIG. 21. Combustion Stand.

Heating Unit. Either a gas burner or an electric heating unit [2] may be employed. The long gas burner (Fig. 20) is approximately 17 cm. long and should be adjustable in height. Instead of this long burner, which consists of a flame distributor mounted on a gas burner, a detachable adapter, which serves equally well as a flame distributor and can be mounted on any gas burner (Bunsen or Tirrill), is entirely satisfactory.

The combustion stand (Fig. 21) ordinarily used in conjunction with the long burner is about 27 cm. long and 20 cm. high up to the V-shaped notches in the center brace at the ends of the stand. The two L-shaped side railings of the stand carry a separate U-shaped wire tunnel of the same length as the long burner to deflect the heat downward upon the combustion tube. The stand is also provided with a Transite plate which is riveted to an adjustable brace in front of the stand. For the movable burner a Bunsen or Tirrill burner is preferred. Another Transite or asbestos plate, 30 cm. high and 15 cm. wide and provided with an opening for the combustion tube, is fastened to the stand to protect the nitrometer from the heat emanating from the heating unit. If an electric heating unit is employed, the stand on which the combustion tube rests is usually an integral part of the furnace and consequently may vary in shape or construction, but essentially it should be similar to the stand described above.

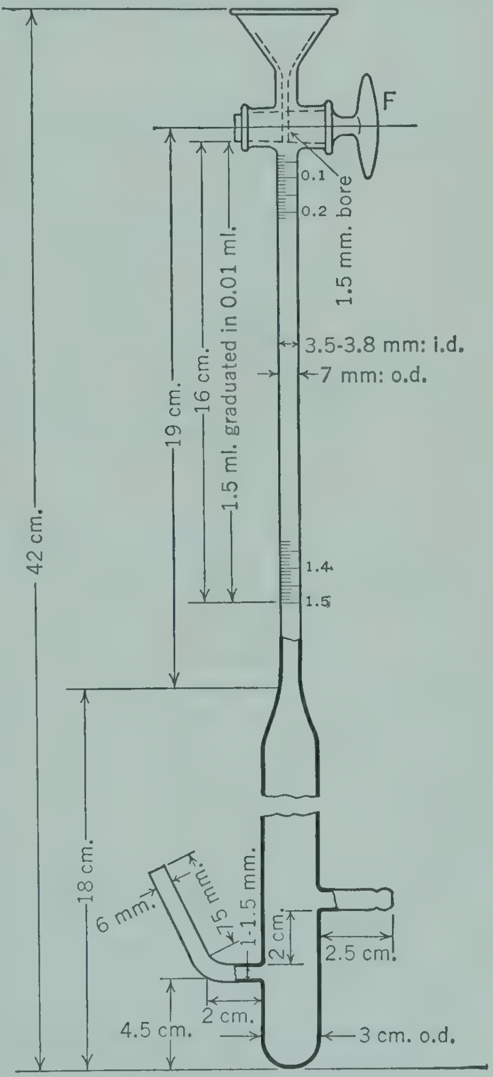


Fig. 22. Nitrometer.

Nitrometer (Fig. 22). The nitrometer or azotometer in which the nitrogen liberated during the combustion is collected is provided with a metal stand and a separate two-way stopcock. Its measurements should closely conform to the specifications given in Fig. 22, because deviations invariably lead to difficulties and inaccuracies which may render the instrument practically worthless. The apparatus measures approximately 42 cm. over all; the upper, calibrated part is about 16 cm. long and has an outside diameter of 7 mm. and an inside diameter of 3.5 to 3.8 mm. The apparatus is calibrated for 1.5 ml. with graduations to 0.01 ml.; the 0.001 ml. are estimated with the aid of a suitable magnifying device attached to the stem of the instrument. It is very important that the buret of the nitrometer widen gradually to the lower part like the neck of an Erlenmeyer flask to prevent the congestion of the gas bubbles at this point. This lower section of the nitrometer is about 18 cm. long and has a diameter of about 3 cm. Approximately 4 cm. from the bottom is a gas inlet, and 2 cm. above it on the opposite side is a side arm which is connected to the leveling bulb with alkali-resistant rubber tubing. The gas inlet tube enters the nitrometer horizontally, but ascends 2 cm. beyond at an angle of 45 degrees. Its inside diameter should be between 1 and 1.5 mm., but should not exceed 1.5 mm., because the size of the microbubbles and consequently also the duration of the combustion depend upon the inside diameter of this inlet tube. The two-way stopcock *E* (Fig. 23) is sealed to the nitrometer or attached with a heavy rubber tubing; the capillary outlet facing the combustion train is bent horizontally at the proper height to align with the capillary end of the combustion tube when the tube rests on the combustion stand. The stopcock has an oblique bore leading to the nitrometer and a downward outlet which permits the carbon dioxide to pass to the outside when sweeping out the air from the combustion tube before the combustion. Two fine grooves about 2 mm. long, etched into the end of the bore in opposite directions, permit a more accurate adjustment of the flow of the gases into the nitrometer.

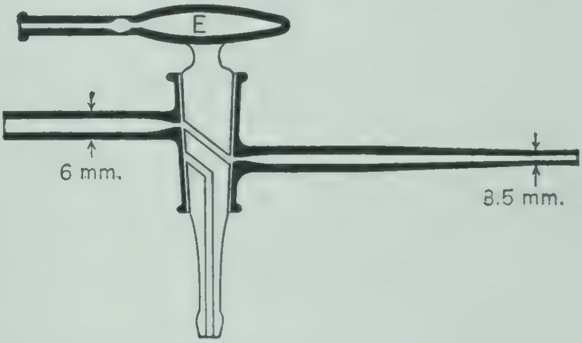


FIG. 23. Stopcock Leading from Combustion Tube to Nitrometer.

Before being used and whenever necessary thereafter, the nitrometer is cleaned with cleaning solution, then rinsed well with tap water, distilled water, and alcohol, and placed upside down to dry. The stopcocks and rubber tubings, however, are removed and cleaned separately. The rubber tubing connecting the leveling bulb to the nitrometer is washed with 50% potassium hydroxide solution when necessary, and never with water. When clean and dry, the nitrometer is filled with mercury up to 1 mm. of the side arm which connects it with the leveling bulb by placing the mercury in the leveling bulb and letting it run slowly into the nitrometer. The leveling bulb is filled with 50% potassium hydroxide solution to two-thirds of its capacity and placed in an open ring clamp at about the same height as the side arm. A ring clamp attached to a metal clamp holds the nitrometer loosely in place. Before the nitrometer is connected to the combustion tube with a rubber tubing of about 4-cm. length, 9-mm. outside diameter and 2-mm. bore, stopcock *E* is sparingly but nevertheless well lubricated with special stopcock grease.

Calibration Table. The calibration table expresses the correct volume to 0.001 ml. for every 0.01 ml. of the graduation of the nitrometer. It is usually supplied with every apparatus, but may also be made in the laboratory. Since the lowest point of the meniscus of the 50% potassium hydroxide solution is read, the calibration of the nitrometer with mercury must be carried out with the instrument in inverted position. Such measurements should be made for every 0.2 ml. beginning with 0.1 up to 0.9 ml., and for every 0.3 ml. thereafter. By interpolating the correct volume for each division (0.01 ml.) of the nitrometer, a chart may be prepared as shown in the table below, which illustrates this type of calibration as recommended by F. Pregl.[43, 44, 47] A graphical chart may also be employed. In such a chart the abscissa carries the milliliters (0.1 to 1.5 ml.), while the deviations in the graduation

PRECISION NITROMETER ACCORDING TO F. PREGL, AT 20° C. (KOH)

ml.	0.00	0.01	0.02	0.03	0.04	0.05	0.06	0.07	0.08	0.09
	Capacity in Milliliters									
0.1	0.099	0.109	0.119	0.129	0.139	0.149	0.159	0.169	0.179	0.189
0.2	0.199	0.209	0.219	0.229	0.239	0.249	0.259	0.269	0.279	0.289
0.3	0.299	0.309	0.319	0.329	0.339	0.349	0.359	0.369	0.379	0.389
0.4	0.399	0.409	0.419	0.429	0.439	0.449	0.459	0.469	0.479	0.489
0.5	0.499	0.509	0.519	0.529	0.539	0.549	0.559	0.569	0.579	0.589
0.6	0.599	0.609	0.619	0.629	0.639	0.649	0.659	0.669	0.679	0.689
0.7	0.698	0.708	0.718	0.728	0.738	0.748	0.758	0.768	0.778	0.788
0.8	0.798	0.808	0.818	0.828	0.838	0.848	0.858	0.868	0.878	0.888
0.9	0.898	0.908	0.918	0.928	0.938	0.948	0.958	0.968	0.978	0.988
1.0	0.998	1.008	1.018	1.028	1.038	1.048	1.058	1.068	1.078	1.088
1.1	1.098	1.108	1.118	1.128	1.138	1.148	1.158	1.168	1.178	1.188
1.2	1.199	1.209	1.219	1.229	1.239	1.249	1.259	1.269	1.279	1.289
1.3	1.299	1.309	1.319	1.329	1.339	1.349	1.359	1.369	1.379	1.389
1.4	1.399	1.409	1.419	1.429	1.439	1.449	1.459	1.469	1.479	1.489
1.5	1.499	.....	.....	.....	.....	.....	.....	.....	.....	.....

form the ordinate. A nitrometer closely following the zero line would indicate perfection, and calibration errors that remain constant would disclose a satisfactory instrument, whereas a nitrometer showing a calibration curve changing repeatedly from plus to minus obviously would be of doubtful value.

Introduction Funnel. For the introduction of copper oxide and substance into the combustion tube a funnel is employed. It is made from a test tube, and its upper section is 4 to 5 cm. long and about 2 cm. in diameter; it is drawn out gradually to a conical capillary 6 to 7 cm. long and 3 to 4 mm. in diameter. This long stem is necessary to prevent particles of fine copper oxide, to which substance may adhere, from settling near the orifice of the combustion tube.

Reagents

Marble. Clean marble, about 2 kg. for each Kipp generator, is broken up into pieces small enough to pass through the side opening of the generating chamber. It is treated previous to its use by washing it with dilute hydrochloric acid (5%), decanting the solution, and then boiling the marble with water in a thick-walled flask for two to three hours, changing the water until it remains clear. To expel the air from the pores of the marble the flask is attached to a water suction pump and evacuated for about thirty minutes; this procedure of boiling and evacuation may be repeated several times. To prevent the reabsorption of air the marble is kept under water until it is placed in the Kipp generator.

Hydrochloric Acid. Four volumes of concentrated hydrochloric acid (sp. gr.: 1.178–1.185) are diluted with one volume of distilled water. About 4 liters of this diluted acid is required for one Kipp generator.

Copper Oxide. Copper oxide in wire form is used; it is 3 to 5 mm. long and about 0.5 in diameter and should be without pores. Porous copper oxide, because of its tendency to retain air, is objectionable; after pre-ignition it should be evacuated in a side-arm test tube and then saturated with carbon dioxide. The fine copper oxide, of 40 to 100 mesh, is obtained by grinding the copper oxide wires and sifting through sieves of 40 to 100 mesh. Both forms of copper oxide are used over again after being re-ignited separately in a nickel or iron dish over a blast flame at 700 to 800° C., or being heated to a dull red for fifteen minutes. Coarse and fine oopper oxide are kept in separate test tubes of about 80-ml. capacity, preferably in an atmosphere of carbon dioxide.

Metallic Copper. Copper wool or copper in wire form serves this purpose. Copper wool, pressed to form a roll about 4 cm. long and of somewhat smaller diameter than the inside diameter of the combustion tube, is effectively reduced by igniting it and placing it, while still hot, in a test tube containing a few milliliters of methyl alcohol. The copper wire is reduced by heating it in an atmosphere of hydrogen.

Mercury. Clean mercury is used for the safety funnel tube of Kipp generator I, for the leveling bulb of the gasometer, and as a sealing liquid in the nitrometer. About 3.5 kg. is needed in all.

Potassium Hydroxide Solution (50%). Potassium hydroxide (c.p. in pellets) is dissolved in an equal weight of distilled water. It is left standing for 24 hours and then is filtered through good fluted filter paper, glass wool, or a Büchner funnel covered with Gooch crucible asbestos. The filtration is repeated until a perfectly clear solution is obtained, which is stored in a rubber-stoppered reagent bottle.

Stopcock Grease. Heavy vaseline with a lanolin base is used as a lubricant for all the stopcocks of the apparatus.

Procedure

Solids. The finely powdered substance is weighed in the weighing tube and transferred to a mixing tube about 7.5 cm. long and 1 cm. in diameter. A Pyrex test tube having these dimensions, but without flare, will serve the purpose. Sufficient substance should be taken to obtain a volume of nitrogen amounting to at least 0.3 ml.

Liquids. The liquid sample is weighed in a sealed capillary pipet.[14] After the introduction of the 5-cm. layer of coarse and the 2-cm. layer of fine copper oxide into the combustion tube, the capillary is centrifuged; its tip is then broken off, and the capillary and the tip are placed in the combustion tube with the open end of the capillary facing the temporary combustion-tube filling; more fine copper oxide is added until the capillary is completely covered.

Viscous oils, thick syrups, waxes, sticky semi-solids, and hygroscopic substances are weighed in a porcelain boat; the substance in the boat is covered with fine copper oxide and then placed in the combustion tube after the temporary combustion-tube filling. Sufficient fine copper oxide is added to cover the boat.

Introduction of the Temporary Filling and the Substance. The combustion tube is detached from the apparatus by first pulling out the gas inlet tube from the rubber stopper, removing the stopper, and then disconnecting the combustion tube from the nitrometer; the rubber connection remains attached to the nitrometer. The temporary filling present is removed by tapping and placed in a wide-mouth reagent bottle. The combustion tube is wiped twice with a cotton wad wound tightly around the roughened end of a stiff iron wire about 40 cm. long. A 5-cm. layer of coarse copper oxide is introduced directly into the combustion tube and is followed by a 2-cm. layer of fine copper oxide which is added by means of the introduction funnel. This constitutes the *temporary* combustion-tube filling. A 2-cm. layer of fine copper oxide, or more for a sample exceeding 5 mg., is added to the substance in the mixing tube, which is tapped to free its mouth from particles of copper oxide, closed with a tight-fitting cork, and then well shaken to mix the substance thoroughly with copper oxide. By tapping the mixing tube, loosening the cork, and then tapping it again, the possibility of losing traces of substance adhering to the cork is prevented. The mixture of fine copper oxide and substance is transferred to the combustion tube with the aid of the introduction funnel. About 1 cm. of fine copper oxide is then placed in the mixing tube, which is again well shaken and the material transferred to the combustion tube as before. To insure the quantitative transfer of the substance this washing is repeated once more, whereupon the funnel as well as the combustion tube are repeatedly tapped to prevent particles of substance from remaining on the upper part of the combustion tube. Finally, a little coarse copper oxide, no more than enough to form a 1- to 1.5-cm. layer, is added directly to assure the accomplishment of this purpose.

Two wire gauzes 5 cm. long for the movable burner and one 19 to 20 cm. long for the long burner are placed over the combustion tube for its protection from the flames of the burners; they are slipped over the capillary ending of the combustion tube, with the long wire gauze covering the permanent filling. To keep the temporary filling from being spread in the combustion tube, it is brought into a vertical position, tapped, and then placed on the combustion stand. The mouth of the combustion tube and the capillary end are cleaned with cotton wound around a toothpick or the small knurled iron wire. The capillary ending, the rubber stopper, and the capillary of the glass tube leading to the gasometer are lubricated with a trace of glycerin. The rubber stopper is moistened very sparingly, because if excessively lubricated it may be forced out of the combustion tube when it is placed under pressure, thereby ruining an analysis. The combustion tube is attached to the apparatus by first connecting it glass-to-glass to the nitrometer, then inserting the rubber stopper and through it the capillary of the glass tube of the gasometer in such a way that the tip of the capillary projects not more than 1 mm. beyond the rubber stopper in the mouth of the combustion tube. Finally, the combustion tube is so adjusted that it rests evenly on the combustion stand and the permanent filling at the capillary ending protrudes 5 cm. beyond the long burner. This is necessary for the gradual cooling of the gas stream to prevent possible dissociation of the carbon dioxide to carbon monoxide and oxygen which are not absorbed by the 50% potassium hydroxide solution.

Preparation for the Combustion. Stopcock *F* on top of the nitrometer is sparingly lubricated with the special stopcock grease; the barrel of the stopcock should become transparent, but no grease must enter the stem of the nitrometer, because deposits of grease at that point will not only cause the potassium hydroxide solution to froth but also lead to high results. The nitrometer is filled with 50% potassium hydroxide solution by opening stopcock *F*, raising the leveling bulb until the funnel is about one-fourth filled, then closing the stopcock and placing the leveling bulb in its lower ring clamp. The air is expelled from the combustion tube in the following manner: The gasometer is filled with carbon dioxide from the generating chamber of Kipp II by closing stopcock *D*, opening stopcock *C*, and lowering the mercury leveling bulb, by placing it on the table, until the mercury reaches the lower level in the gasometer; stopcock *C* is then closed, the leveling bulb placed in its open ring clamp, and stopcock *D* opened. Stopcock *E*, between combustion tube and nitrometer, is opened in such a way that the gases pass through the downward outlet into the air. About 100 ml. of carbon dioxide is passed through the combustion tube in this manner at a speed of about 50 ml. per minute, whereupon stopcocks *D* and *E* are closed, the gasometer refilled with carbon dioxide, and the washing-out repeated. After the second washing the long burner is lighted, centered under the combustion tube, and its flame so adjusted that it envelops the tube. The flame should be of sufficient intensity to heat the wire gauzes to a dark cherry red (650 to 700° C.), but excessive heating, because of its destructive effects upon the combustion tube, is to be avoided. Wire tunnels are placed over it, a long one, 19 to 20 cm., for the long burner, and a short one, 5 cm., for the movable burner, to reflect the heat downward. The gasometer is filled once more with carbon dioxide and about 80 ml. of it is passed into the air, after which stopcock *E* is turned cautiously to permit the gases to enter the nitrometer at a rate of two to three bubbles per second. A greater speed not only will result in poor absorption of the carbon dioxide by the potassium hydroxide solution, but also will break the fine film covering the mercury in the nitrometer, resulting in a subsequent sticking of the bubbles to the surface of the mercury. The bubbles rising from the inlet tube should be nearly absorbed by the 50% potassium hydroxide solution and show the characteristics typical of microbubbles; they indicate the purity of the carbon dioxide as well as give assurance that the expulsion of the air from the combustion tube has been complete. Should the gas bubbles not be typical microbubbles the combustion tube is washed once more with about 80 ml. of carbon dioxide, which is passed into the air and the above test repeated. When microbubbles are obtained stopcock *D* is closed, stopcock *E* opened completely to the nitrometer, thus bringing the pressure in the combustion tube in equilibrium with atmospheric pressure. Then the leveling bulb is raised to the height of the funnel of the nitrometer, stopcock *F* opened, the collected gas bubbles forced into the funnel, stopcock *F* closed, and the leveling bulb placed in the lower ring clamp where it remains until the end of the combustion.

First Combustion. The combustion is started with a small flame of the movable gas burner 1 cm. in front of the copper oxide. The flame is gradually increased until it is about 5 cm. high and hisses slightly. As soon as the rapid evolution of gas bubbles, due to the application of additional heat, has subsided, the burner is slowly advanced toward the copper oxide filling, but its movement is controlled so that no more than one gas bubble per second, less rather than more, rises in the nitrometer. Disregard of this precaution leads either to low results, due to incomplete combustion of the substance or insufficient contact of the gases with the metallic copper to insure the reduction to elementary nitrogen of the oxides of nitrogen, which would be absorbed by the 50% potassium hydroxide solution; or to high results, which may be the consequence of the dissociation of the carbon dioxide to carbon monoxide and oxygen. Should the gas bubbles rise more rapidly, then the movable burner is removed until their frequency has returned to normal. The combustion is continued until the entire temporary filling up to the long burner has been thoroughly heated, which requires from twenty to twenty-five minutes. After the part of the combustion tube containing the substance has been passed with the burner, no more gas bubbles may rise and the receding of the mercury into the gas inlet tube of the nitrometer can be observed, indicating that the gaseous combustion products have passed their maximum expansion. The heating may then be speeded up somewhat, but nevertheless the heating must be continued until the permanent filling is reached. For some substances, especially those whose percentage of nitrogen is rather low, no nitrogen may be collected during the first combustion. In this event the time factor is the only guidance.

Second Combustion and Sweeping Out. With the first combustion completed, stopcock *E* is closed and the gasometer filled with carbon dioxide up to the beginning of the graduation. To bring the mercury to the level of the first graduation mark, stopcock D is turned to connect the gasometer with the downward outlet, thus permitting the few milliliters of excess carbon dioxide to escape. Then stopcock D is turned to connect the gasometer with the combustion tube. It is of the utmost importance that stopcock E be opened very cautiously to the nitrometer, so that no more than three gas bubbles in two seconds pass into the nitrometer. Although a groove is etched into the bore of stopcock E to permit easier regulation, nevertheless it is advisable to practice the gradual opening of this stopcock before an analysis is started. To watch the mercury being forced back into the nitrometer may also be helpful, because it is not possible to see when the bore of the plug connects with the opening of the capillary tubing. With the speed so regulated that only three gas bubbles in two seconds rise in the nitrometer, the movable burner is placed once more in front of the temporary filling and the combustion repeated, only more rapidly, taking about ten minutes to heat the entire temporary filling up to the long burner. The movable burner is then extinguished, and, when the gas bubbles have become considerably smaller, their speed may be increased to two or three per second. When their size is again reduced to microbubbles, indicating that all the nitrogen has been washed out by the carbon dioxide, the rate of speed may be increased to five per second and the long burner extinguished. Fifty milliliters of carbon dioxide is usually sufficient for the sweeping out of the combustion gases, and when the mercury has reached the graduation mark in the gasometer indicating this volume, stopcock D is closed and stopcock E gradually opened completely until no more gas bubbles rise, thus again establishing the equilibrium with atmospheric conditions existing before the start of the combustion. Stopcock E is then closed and stopcock D opened again to permit the combustion tube to cool in an atmosphere of carbon dioxide. Failure to close stopcock E during the cooling of the combustion tube will cause the mercury in the nitrometer to be drawn into the combustion tube, thereby spoiling the filling and necessitating its replacement.

Determination of the Volume of Nitrogen. The leveling bulb is raised to the level of the 50% potassium hydroxide solution in the nitrometer by adjusting the upper ring clamp to the proper height. Gas bubbles remaining undissolved on the meniscus of the 50% potassium hydroxide solution in the nitrometer may be broken up by placing the leveling bulb in the lower ring clamp and striking the rubber tubing with the edge of the hand. After an interval of ten minutes, to permit the 50% potassium hydroxide solution to run down the inside wall of the nitrometer, the volume of nitrogen collected is read to the nearest 0.001 ml. with the aid of the magnifying lens. The barometric pressure is also read at this time to within ±1 mm. and the temperature of the 50% potassium hydroxide solution determined within ±0.5° C. by placing a thermometer in the funnel of the nitrometer and then in the leveling bulb. If the two readings do not coincide their average is taken.

Determination of the Air and Adsorption Error. Since the carbon dioxide supplied by the Kipp generator and the copper oxide used for the temporary filling always contain some residual air, which would cause high results, this air and absorption error must be determined and deducted from the total volume of nitrogen collected. The magnitude of this error, or, in other words, the amount of air contained in a given volume of carbon dioxide, 50 ml. in this case, is determined by combusting any substance which is free of nitrogen. However, it is not necessary to weigh this sample, although it should be approximately between 3 and 5 mg., but it is mixed with fine copper oxide as described and the combustion tube filled as for a regular determination. However, since not enough nitrogen will be collected from the residual air in 50 ml. of carbon dioxide to reach the calibration in the nitrometer which begins at 0.050 ml., some nitrogen is left in the stem from a previous determination, or air is let in by simply closing stopcock *F* when the 50% potassium hydroxide solution has reached a level between 0.150 and 0.200 ml. Ten minutes after the leveling bulb has been adjusted to the height of the meniscus the volume is read to the nearest 0.001 ml.; the temperature of the 50% potassium hydroxide solution and the barometric pressure are also taken at this time. The air is driven out of the combustion tube as described, except that for the testing of microbubbles not more than 1 ml. of carbon dioxide is used. The combustion and sweeping out of the gases with 50 ml. of carbon dioxide is carried out in exactly the same manner as in a regular determination. With the combustion completed, the leveling bulb is placed up to the meniscus of the 50% potassium hydroxide solution in the nitrometer, and ten minutes later the volume, temperature, and barometric pressure are determined. After the necessary corrections for possible temperature and pressure changes have been made, the difference between the reading before the combustion and after its completion represents the sum of the air and absorption error from the carbon dioxide and copper oxide. This error should be constant and vary no more than ±0.002 ml. even over rather long periods of time; consequently, once this constancy has been established, it is not necessary to determine the air error every time the apparatus is used. An air and absorption error of 0.050 ml. for 50 ml. of carbon dioxide is tolerable, but a correctly set up apparatus will give an air and absorption error of 0.010 to 0.015 ml. for 50 ml. of carbon dioxide.

Time:	*Minutes*
Weighing of the sample	10
Filling and sweeping out the combustion tube	15
First combustion	30
Second combustion	10
Sweeping out of combustion products	30
Total	95

Calculation:

Determination of the Net Volume of Nitrogen:

Collected ml. of nitrogen (nitrometer reading),
Minus, or plus, calibration correction (see nitrometer certificate),
Minus air and adsorption error (determined by blank analysis),
Minus 1.1% of total collected ml. of nitrogen (0.5% for adhesion of the 50% potassium hydroxide solution to the wall of the nitrometer, 0.3% for the vapor pressure of the 50% potassium hydroxide solution,* 0.3% for the temperature reduction of the barometric reading from room temperature to 0° C.) [55,56]

Instead of the above detailed corrections an overall correction of 2% may be deducted from the nitrometer reading already corrected for calibration errors.[43, 44]

* This pressure correction may be subtracted directly from the barometer reading.[34]

Percentage of Nitrogen:

Log of net volume of nitrogen,
Plus log of 1 ml. nitrogen at temperature and pressure collected (see nitrogen reduction tables on pp. 301-310),
Plus negative log of weight of substance;
Antilog of total = percentage of nitrogen.

Remarks

The micro Dumas method as described here differs from the original procedure of F. Pregl [43, 44, 47] mainly because of the introduction of a gasometer in the apparative arrangement by J. B. Niederl and co-workers.[38, 55, 56] The volume of carbon dioxide is measured and kept constant for every combustion, thereby eliminating the dependence upon the ill-defined microbubbles. C. Weygand [58] and others [37, 38, 55, 56] have shown that the size of the microbubbles—all other experimental conditions remaining the same—depends upon the inside diameter of the gas inlet of the nitrometer and consequently may vary with different instruments. Furthermore, measurements with the gasometer show that the time required for the passing of a definite volume of carbon dioxide into the nitrometer while the same speed of the bubbles (one bubble per second) is maintained also depends largely upon the diameter of this gas inlet. It has been experimentally established that, whereas 30 ml. of carbon dioxide could be passed into one nitrometer in twenty minutes by maintaining a rate of speed of one bubble per second, it required thirty minutes to pass the same amount of gas at the same rate of speed into another nitrometer having a narrower gas inlet. In the procedure described here the end of the combustion is given by the exhaustion of a definite volume of carbon dioxide in the gasometer; in the method of F. Pregl [43, 44, 47] the end of the determination is given either by a time limit (thirty to forty minutes), also recommended by R. T. K. Cornwell,[11] or by the reappearance of microbubbles, which, according to F. Böck and K. Beaucourt,[5] is an unreliable criterion. The empirical deduction of 2% from the volume of nitrogen as suggested by F. Pregl has been investigated by O. Trautz [55] and was found to consist of several factors, some of which are constant while others are subject to variation, as for instance the so-called air error, a circumstance which led to the adaptation of the calculation formula presented herein. A modified gasometer has been described by J. G. Sandza and J. F. Alicino.[49]

A survey of the literature reveals that all phases and integral parts of the apparatus have been thoroughly investigated.[17a] The open Kipp generator, as used by F. Pregl, has been largely discarded in favor of arrangements protecting the contents of the Kipp generator from contact with air.[12, 24, 39, 51] A self-sealing carbon dioxide generator has been described by F. Hein [24] and E. J. Poth.[42] The same purpose is accomplished by connecting two Kipp generators as described by S. Ogawa [39] and E. Diepolder,[12] which is particularly useful for double set-ups.[20] The arrangement described not only protects the contents of the Kipp generator delivering the carbon dioxide from contact with air but also permits convenient evacuation of the system and easy replacement of the expended acid with fresh acid whenever necessary without disturbing the air-tight connections of the apparatus. Thus such an arrangement needs to be dismantled only for the initial introduction of the marble, which is prepared according to the procedure of F. Pregl [43, 44, 47] and A. Friedrich.[17] Since the flushing out of the Kipp generator, as described by F. Pregl, is no longer necessary, such an apparatus will last for more than a thousand analyses.

Replacement of the Kipp generator as a source of supply of carbon dioxide by magnesium carbonate has been suggested by a number of investigators.[13, 19] W. S. Ide [28] has demonstrated the successful application of the semi-micro magnesite method of E. Berl and H. Burkhart [3] to milligram samples. This method, however, results in a much higher air error and also requires a complete new filling of the combustion tube for every determination. The use of fused sodium and potassium carbonate has been recommended by A. Meixner and F. Kröcker.[33] The use of sodium bicarbonate has been suggested by J. V. Dubsky [13] and others, but H. Fischer[15] found that it does not offer any advantages over the preparation of carbon dioxide from marble. F. Breuer[6] described an exchangeable carbon dioxide generator using sodium bicarbonate and the gasometer arrangement of J. B. Niederl and co-workers.[38, 56] The use of solid carbon dioxide has been suggested for macro Dumas nitrogen determinations by F. Shea and C. E. Watts[52] and for micro Dumas nitrogen determinations by E. B. Hershberg and G. W. Wellwood.[26] The last two have also devised for this purpose a suitable generator with adequate safety valve arrangements.

The apparatus (Fig. 24) consists of the dry-ice storage bottle (*a*), which is usually an ordinary commercial vacuum bottle of about 1-liter capacity, the thick-walled delivery tube (*b*), provided with a safety valve arrangement (*c*). The thick-walled vertical portion of the delivery tube (*b*) is 9 cm. long, its outer diameter is 9 mm., and its inner is 5 mm.; it extends through a tight-fitting rubber stopper into the dry-ice supply bottle (*a*). Its right horizontal side arm is 20 cm. long and of the same thickness and diameter; it carries a stopcock (*d*) and is connected to the combustion tube. The horizontal side arm at the left is of the same thickness and diameter. At a distance of 6 cm. from the vertical arm is the safety device. This consists of a vertical mercury U-tube manometer (*e*), which is 17 cm. long and is also of the same thickness and inner diameter. Eight centimeters below the horizontal arm the descending arm carries a paper diaphragm (*f*) for the release of the excess pressure.

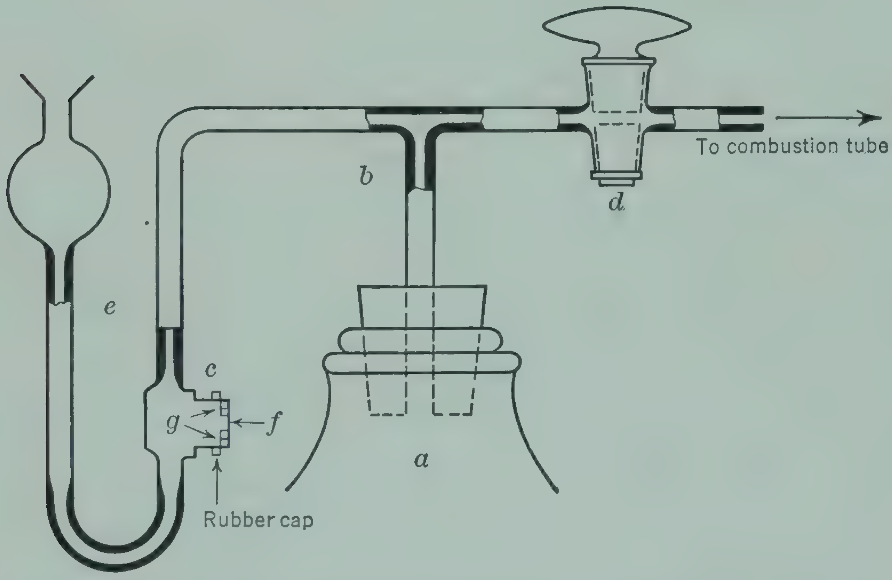


FIG. 24. Dry Ice Carbon Dioxide Generator.[26] Explanation in Text.

The best pressure regulation is obtained by using as a membrane hardened filter paper of such porosity that the mercury cannot diffuse. It is advisable to insert a rubber washer (*g*) between the glass and the paper membrane to form a tight seal. When this is properly adjusted the fluctuation of the pressure is claimed to be less than 1 mm. After the supply bottle has been filled with dry ice, the apparatus is allowed to stand over night. The escaping carbon dioxide gas will sweep out all the air from the apparatus, and good microbubbles usually result the next day. A charge of about 1 pound of dry ice is claimed to last about 100 hours. For continuous operation the generator is recharged every fourth or fifth evening. Substitution of this type of generator for the Kipp generator arrangement has been found entirely satisfactory. Since the gasometer cannot be used in this type of generator, a time factor has to be introduced for the sweeping-out process and the empirical correction of F. Pregl [43, 44, 47] of 2% surplus gas has to be applied in place of the detailed correction introduced by J. B. Niederl and co-workers.[38, 56]

In regard to the combustion tube filling, with the exception of the suggestion to use iodine pentoxide instead of the metallic copper,[46] few changes have been reported and the original Pregl filling and arrangement have been found quite satisfactory,[8, 9, 13, 16, 18, 21, 25, 31, 41, 54, 55, 57] as well as the combustion procedure and arrangement of the nitrometer.[13, 32] A new nitrometer, featuring the elimination of the top stopcock, has been described by R. T. Milner and M. S. Sherman.[34]

Occasionally substances are encountered which give low nitrogen values.[17, 23, 34, 47, 48, 53] In dealing with them the addition of copper acetate to the sample has been suggested by D. F. Hayman and S. Adler.[23] Introduction of potassium chlorate into the combustion tube has been recommended by J. R. Spies and T. H. Harries.[53] The sticking of the gas bubbles to the surface of the mercury in the nitrometer was found to be due to such factors as too narrow an opening of the gas inlet of the nitrometer, too short a distance between this gas inlet and the level of the mercury, and excessive greasing of the stopcock connecting the combustion tube with the nitrometer. With the elimination or correction of these defects this difficulty is readily overcome; the suggestions of F. Pregl,[43, 44, 47] O. Trautz,[55] B. Flaschenträger,[16] and C. Weygand [58] that the surface of the mercury be dusted with powdered copper oxide have proved helpful. M. L. Nichols [36] recommends mercurous oxide for the same purpose.

Other innovations include: all-glass apparatus without any rubber connections whatsoever; [35, 55] electric combustion furnaces; [2] simultaneous determination of nitrogen and carbon and hydrogen; [4] replacement of the nitrometer stopcock by a needle valve;[27] and placement of a screw adjustment on the elongated handle of this stopcock.

Since in the Dumas nitrogen method the reaction product is always determined volumetrically, the method without further modification, except for a slower combustion of the larger sample (8–15 mg.), lends itself most admirably for general use without a microanalytical balance,[29, 30, 37, 38, 50] as recently demonstrated by J. B. Niederl and co-workers.[37] Earlier attempts involved solution or dilution methods.[38, 50]

The development of semi-micro methods[7, 10, 40] is usually governed by a similar desire, i.e., to eliminate microanalytical balances, and either comprises mere enlargement of the original Pregl micro Dumas apparatus, or combustions in vacuum[7, 40] with either volumetric or manometric determination of the nitrogen.

LITERATURE

1. Allen, C. F. H., and Young, D. M., *Can. J. Research*, **14**, Sect. B, 216 (1937).
2. Beazley, C. W., *Ind. Eng. Chem., Anal. Ed.*, **10**, 605 (1938).
3. Berl, E., and Burkhart, H., *Ber.*, **59**, 897 (1926).
4. Berraz, G., *Anales investigaciones cient. tecnol.* (*Argentina*), **7**, 70 (1937).
5. Böck, F., and Beaucourt, K., *Mikrochemie*, **6**, 69, 202 (1928).
6. Breuer, F., *Ind. Eng. Chem., Anal. Ed.*, **9**, 354 (1937).
7. Brodie, S. S., and Natelson, S., *Ind. Eng. Chem., News Ed.*, **17**, 521 (1939).
8. Brody, E., and Millner, T., *Z. anorg. Chem.*, **164**, 86 (1927).
9. Cherbuliez, E., *Helv. Chim. Acta*, **3**, 652 (1920).
10. Clark, E. P., *J. Assoc. Official Agr. Chem.*, **16**, 255 (1933).
11. Cornwell, R. T. K., *Ind. Eng. Chem., Anal. Ed.*, **4**, 42 (1932).
12. Diepolder, E., *Chem. Ztg.*, **43**, 355 (1919).
13. Dubsky, J. V., "Vereinfachte quantitative Mikroelementaranalyse organischer Substanzen," Veit and Co., Leipzig, 1917; *Ber.*, **50**, 710 (1917).
14. Eigenberger, E., *Mikrochemie*, **26**, 273 (1939).
15. Fischer, H., *Ber.*, **51**, 1322 (1918).
16. Flaschenträger, B., *Mikrochemie*, **8**, 1 (1930).
17. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, pp. 55–75; (*a*) *Mikrochemie*, **10**, 355 (1932).
18. Funck, C., "Mikroanalyse nach der Mikro-Dennstedt-Methode," Munich, 1925.
19. Govaert, F., *Mikrochemie*, **9**, 338 (1931).
20. Graenacher, Ch., *Helv. Chim. Acta*, **2**, 81 (1919).
21. Halla, F., *Mikrochemie*, **7**, 202 (1929).
22. Hamill, W. H., and Alicino, J. A., *Ind. Eng. Chem., Anal. Ed.*, **9**, 290 (1937).
23. Hayman, D. F., and Adler, S., *Ind. Eng. Chem., Anal. Ed.*, **9**, 197 (1937).
24. Hein, F., *Z. angew. Chem.*, **40**, 864 (1927).
25. Hernler, F., *Mikrochemie, Pregl Festschrift*, 1929, p. 143.
26. Hershberg, E. B., and Wellwood, G. W., *Ind. Eng. Chem., Anal. Ed.*, **9**, 303 (1937).

27. Hershberg, E. B., and Southworth, L., *Ind. Eng. Chem., Anal. Ed.*, **11**, 404 (1939).
28. Ide, W. S., *Ind. Eng. Chem., Anal. Ed.*, **8**, 56 (1936).
29. Kraemer, G., *J. prakt. Chem.*, (2) **97**, 59 (1918).
30. Lauer, W. M., and Sunde, C. J., *Mikrochemie, Pregl Festschrift*, 1929, p. 235.
31. Limprecht, H., *Ann.*, **108**, 46 (1858).
32. Lucas, R., and Grassner, F., *Mikrochemie*, **6**, 124 (1927).
33. Meixner, A., and Kröcker, F., *Mikrochemie*, **5**, 125 (1927).
34. Milner, R. T., and Sherman, M. S., *Ind. Eng. Chem., Anal. Ed.*, **8**, 331 (1936).
35. Mueller, E., *Mikrochemie*, **5**, 35 (1927).
36. Nichols, M. L., *Ind. Eng. Chem., Anal. Ed.*, **5**, 149 (1933).
37. Niederl, J. B., Niederl, V., Nagel, R. H., and Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).
38. Niederl, J. B., Trautz, O., and Saschek, W., *Mikrochemie, Emich Festschrift*, 1930, p. 219.
39. Ogawa, S., *Science Repts., Tôhoku Imp. Univ.*, **16**, 667 (1927).
40. Pentschev, N. P., *Z. anal. Chem.*, **113**, 431 (1938).
41. Perrot, A., *Compt. rend.*, **48**, 53 (1859).
42. Poth, E. J., *Ind. Eng. Chem., Anal. Ed.*, **3**, 202 (1931).
43. Pregl, F., and Fyleman, E., "Quantitative Organic Microanalysis," P. Blakiston's Son and Co., Philadelphia, Pa., 1924, pp. 72–94.
44. Pregl, F., and Roth, H., "Die quantitative organische Mikroanalyse," J. Springer, Berlin, 1935, pp. 84–105.
45. Rangaswami, S., *Proc. Indian Acad. Sci.*, **8-A**, 240 (1938).
46. Ronzio, A. R., *Ind. Eng. Chem., Anal. Ed.*, **8**, 122 (1936); **12**, 303 (1940).
47. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son and Co., Philadelphia, Pa., 1937, pp. 69–89.
48. Roth, H., *Mikrochemie, Molisch Festschrift*, 1936, p. 375.
49. Sandza, J. G., and Alicino, J. F., *Ind. Eng. Chem., Anal. Ed.*, **12**, 776 (1940).
50. Saschek, W., *Ind. Eng. Chem., Anal. Ed.*, **3**, 198 (1931).
51. Schoeller, A., *Z. angew. Chem.*, **34**, 586 (1921).
52. Shea, F., and Watts, C. E., *Ind. Eng. Chem., Anal. Ed.*, **11**, 333 (1939).
53. Spies, J. R., and Harries, T. H., *Ind. Eng. Chem., Anal. Ed.*, **9**, 304 (1937).
54. Tiedcke, C., *Mikrochemie*, **16**, 185 (1934); **28**, 76 (1939).
55. Trautz, O., *Mikrochemie*, **9**, 300 (1931).
56. Trautz, O., and Niederl, J. B., *Ind. Eng. Chem., Anal. Ed.*, **3**, 151 (1931).
57. Vetter, F., *Mikrochemie*, **12**, 102 (1935).
58. Weygand, C., "Quantitative analytische Mikromethoden der organischen Chemie in vergleichender Darstellung," Akademische Verlagsgesellschaft, Leipzig, 1931, pp. 24–28.

V. CARBON AND HYDROGEN DETERMINATION

Principle

A weighed amount of the organic compound is combusted in a measured volume of oxygen under controlled pressure to yield quantitatively carbon dioxide from the carbon and water from the hydrogen, the amount of these combustion products being determined gravimetrically. The combustion tube filling is so arranged that other elements present in the organic compound will not interfere with the final quantitative absorption of carbon dioxide and water by suitable absorption tubes containing appropriate absorbing agents.

$C + O_2 \rightarrow CO_2$ (absorbed by *Ascarite* [sodium hydroxide on asbestos])

$H_2 + \frac{1}{2}O_2 \rightarrow H_2O$ (absorbed by *Anhydrone* [magnesium perchlorate])

Apparatus

The combustion train (Fig. 25) consists of the oxygen tank, the pressure regulator I, the drying tube II, the preheater III, the bubble counter and U-tube IV, the combustion tube V, the heating unit VI, the heating mortar VII, the water absorption tube VIII, the carbon dioxide absorption tube IX, the safety tube X, and the Mariotte flask XI.

Oxygen Tank. A standard commercial oxygen tank is used; it is provided with a pressure gauge and reduction valve to facilitate the control of the flow of oxygen.

Pressure Regulator (Fig. 25, I). The pressure regulator is connected to the oxygen tank by means of rubber tubing; it consists of two parts: the outer vessel or bottle, which is 21 to 22 cm. high and has a diameter of 4.5 to 5 cm., and the jacket with an inner tube 22 cm. long and 5 mm. in diameter. The bottle has a wooden head with two metal strips which hold the jacket in place, but nevertheless permit easy adjustment. The jacket has an inlet tube bent at right angles about 1 cm. above the point where it is sealed to the top of the jacket. This inlet tube extends the whole length of the jacket and about 5 mm. beyond its rim at the bottom. An outlet tube is sealed onto the jacket 1 cm. below its top

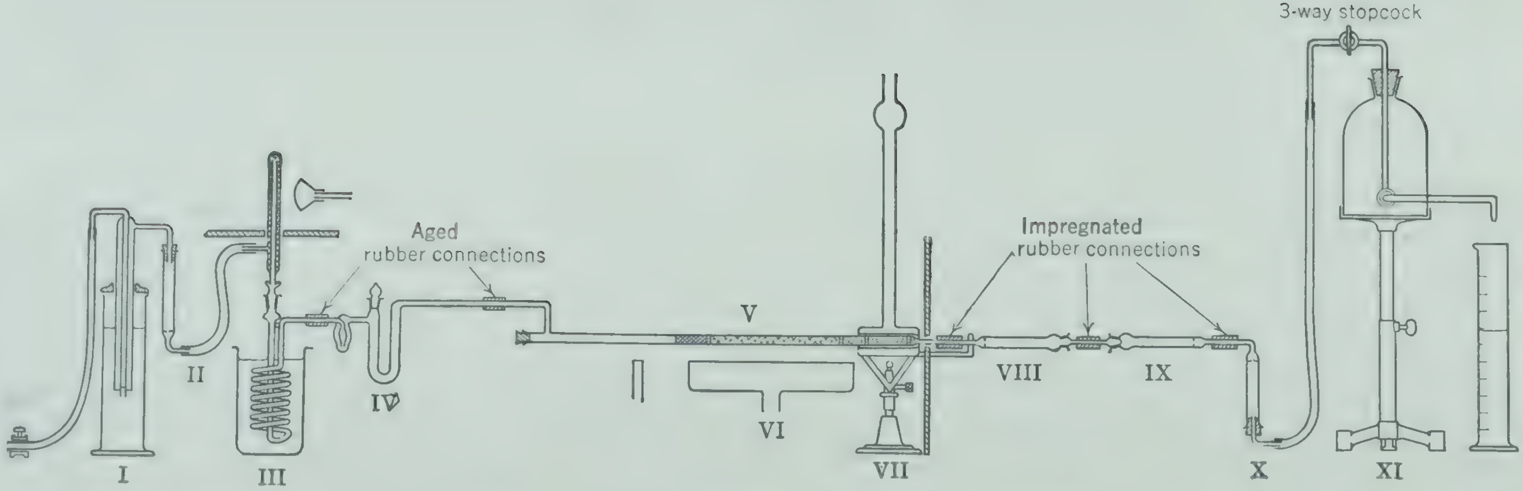

3-way stopcock
Aged
rubber connections
Impregnated
rubber connections
I
II
III
IV
V
VI
VII
VIII
IX
X
XI

Fig. 25. I, Pressure Regulator; II, Drying Tube; III, Preheater; IV, Bubble Counter and U-Tube; V, Combustion Tube; VI, Heating Unit; VII, Heating Mortar; VIII, Water Absorption Tube; IX, Carbon Dioxide Absorption Tube; X, Safety Tube; XI, Mariotte Flask.

and extends horizontally about 4 cm. beyond it, where it is bent downward at right angles. This vertical arm of the outlet tube carries a drying tube filled with Anhydrone (Fig. 25, II). A rubber tubing 30 cm. long, which connects it to the preheater, is attached to the capillary outlet of the drying tube.

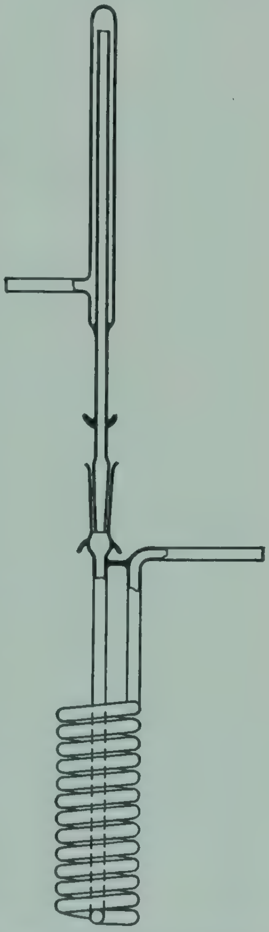


Fig. 26. Preheater.

Preheater (Fig. 26). This device serves the purpose of combusting organic impurities present in the oxygen or given off by the rubber tubings through which it passes. It is about 30 cm. long over all and consists of a quartz combustion tube and a spiral of Pyrex glass which is connected to the combustion tube by a ground-glass joint. The diameter of the spiral tubing below the ground-glass joint is 5 mm. The combustion tube has a total length of about 16 cm. including the ground-glass joint and has a diameter of approximately 1 cm. The combustion tube proper is 10 cm. long and has an inner tube 9 cm. long and is 4 to 4.5 mm. in outside diameter. This inner tube is fused to the combustion tube about 6 cm. above the end of the ground-glass joint. An inlet tube of 4-cm. length and 6-mm. outside diameter is sealed to the combustion tube about 9 cm. from the top. The glass spiral is approximately 14 cm. long, and its coils have a spread of 3 to 4 cm. It ends in a vertical outlet tube which is bent horizontally at about the height of the ground-glass joint or 11 to 12 cm. from the bottom of the spiral. The horizontal arm of the outlet tube is about 8 cm. long and has an outside diameter of 5 to 5.5 mm., corresponding exactly to the outside diameter of the capillary tube of the bubble counter with which, when set up, it must form a glass-to-glass connection. The oxygen enters the preheater through the inlet tube, then passes through the heated copper oxide filling of the combustion tube into the inner tube and downward through the spiral which is immersed in a beaker containing water to cool the oxygen. The combustion tube is filled through the inlet tube with copper oxide in wire form about 2 mm. long and is heated by an electric heating cap or by a gas burner having a fishtail attachment for efficient heat distribution. As a rule, the copper oxide will last as long as the preheater itself, but when necessary it is removed by repeatedly tapping the combustion tube to loosen it and to enable it to fall through the inlet tube. Pieces of copper oxide which have become fused together and cannot be removed otherwise are dissolved by immersing the combustion tube in a solution of concentrated hydrochloric and nitric acid (1 : 1). Before being used again, or when new, the preheater is cleaned with cleaning solution, washed repeatedly with water, distilled water, rinsed with alcohol, and dried first on the suction pump and then in the drying oven at about 120° C.

Bubble Counter and U-Tube (Fig. 27). The purpose of the bubble counter is to provide a means of observing and checking the speed of the oxygen passing through the combustion train. The U-tube, of which the bubble counter is an integral part, is intended to absorb the carbon dioxide and water after the organic impurities present in the oxygen have been combusted by the preheater. An inlet tube of 3-cm. length and 5- to 5.5-mm. outside diameter is fused to the pear-shaped bubble counter, which is about 5 cm. long and 2 cm. in diameter. Inside the bubble counter the inlet tube is bent downward, expands to an outside diameter of 9 to 10 mm., and ends in a capillary 12 to 14 mm. long which has an aperture of 1-mm. maximum inside diameter; this capillary extends within 1 to 2 mm. of the bottom of the bubble counter. An outlet tube of about 2.5-cm. length and 5- to 6-mm. outer diameter, fused to the bubble counter opposite the inlet tube, forms a connection with the U-tube, to which it is sealed about 2 cm. below the rim of the ground-glass stopper. The U-tube is approximately 11 cm. long, and each arm has an outside diameter of 13 mm. It is provided with a capillary outlet tube of 8- to 9-cm. length, 5- to 5.5-mm. outside and

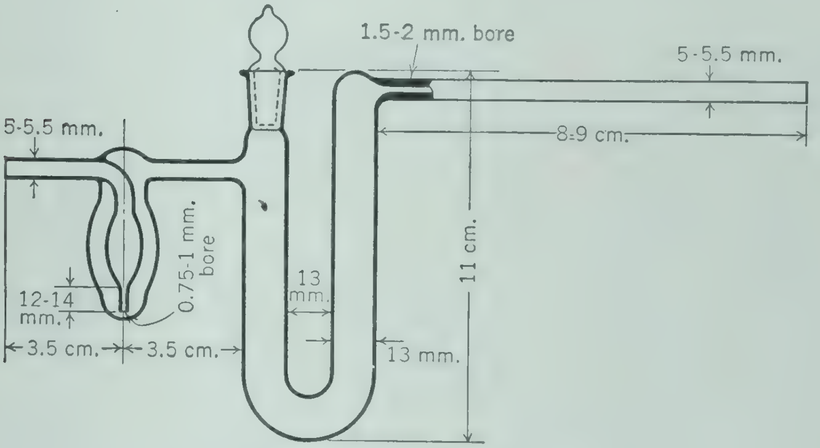


FIG. 27. Bubble Counter and U-Tube.

1.5- to 2-mm. inside diameter, or the same dimensions as the side arm of the combustion tube, with which, when attached, it forms a glass-to-glass connection.

After the bubble counter and U-tube has been cleaned with cleaning solution, washed with water and distilled water, and rinsed with alcohol, it is first dried under suction and then in a drying oven at about 120° C. The U-tube is filled as follows: A plug of clean, dry pliable glass wool is placed against the top of the sealed arm by means of a pliable wire, to prevent particles of Anhydrone from falling into the outlet tube. This arm of the U-tube is filled to its lower bend with Anhydrone which has been sifted through a sieve of 40 mesh to remove the powdery particles. Another plug of glass wool is pressed lightly against the Anhydrone, and the other arm of the U-tube is filled with Ascarite within 2 cm. of the inlet tube. A third plug of glass wool is placed against the Ascarite, and next to that a 2-cm. layer of coarse Anhydrone, intended to absorb most of the moisture present in the oxygen, which otherwise would soon clog up the Ascarite filling. The remainder of the U-tube is filled with pliable glass wool which prevents

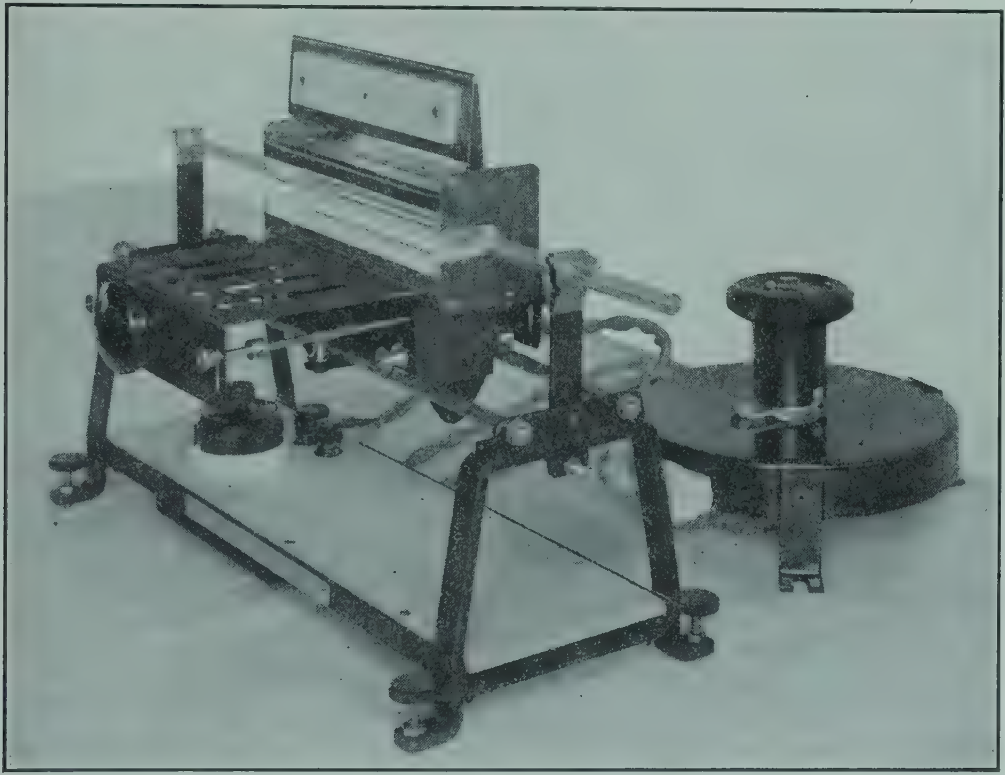


FIG. 28. Electric Combustion Furnace (American Platinum Works).

shifting of the filling and precludes the possibility of Anhydrone falling into the horizontal tube connecting it with the bubble counter. To seal the U-tube the ground-glass stopper is warmed, covered with a little Krönig's glass cement and, while still warm, firmly fixed in place by turning it until the ground-glass joint has become transparent. Finally, enough concentrated sulfuric acid is introduced into the bubble counter with a pipet or capillary, and slight suction is applied from the outlet tube of the U-tube, so that the liquid extends 3 to 4 mm. above the capillary tip.

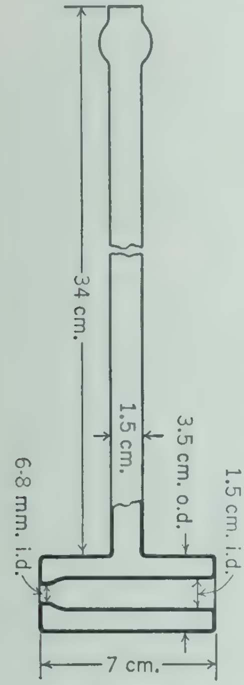


FIG. 29. Heating Mortar.

Heating Unit (Fig. 25, VI). Either a long gas burner or an electric heating unit may be employed, although the latter, for reasons of convenience and uniform heat distribution, is to be preferred. The long burner and combustion stand are of the same type as those for the Dumas apparatus (p. 84). For the movable burner a Tirrill or a Bunsen burner is preferable, because it has the advantage of permitting visual observation of the behavior of the substance during the combustion. The combustion tube is protected from the flames of the burners by pieces of wire gauze about 18 cm. long for the long burner and 5 cm. for the Tirrill or the Bunsen burner. If an electric heating unit is employed then the stand upon which the combustion tube rests is usually an integral part of the furnace and consequently may vary in shape or construction, but essentially it should be similar in its measurements to the stand shown in Fig. 21. A platinum-wired electric furnace shown in Fig. 28,[3, 55] which is movable in all directions, has been found very durable and efficient. Electric furnaces with automatic combustion arrangements are also known.[48, 62, 84, 117]

Heating Mortar (Fig. 29). The latest type of heating mortar is a one-piece, all-glass apparatus. It is about 7 cm. long, 3.5 cm. in diameter, and has a reflux column 34 cm. high and 1.5 cm. in diameter with a bulb-shaped enlargement approximately 1 cm. from the top. The cylindrical chamber or jacket containing the constant-boiling liquid has a horizontal bore of 1.5-cm. diameter with a constriction at one end having an opening 6 to 8 mm. in diameter. This opening permits the passage of the capillary end of the combustion tube, but prevents the combustion tube itself from passing through. The cylindrical chamber rests on a curved metal plate and is fastened to the metal stand with two adjustable metal bands. It is filled about two-thirds with *p*-cymene (b.p. 175–178° C.) and is heated by a microburner which is an integral part of the stand. A heavy copper wire approximately 6 cm. long and 3 mm. thick fits into an opening of the metal plate, where it is also heated by the microburner. This copper wire is placed over the capillary constriction of the water absorption tube during the combustion.

Combustion Tube (Fig. 30). The combustion tube may be of Supremax glass, Pyrex glass 172, or quartz; if of quartz it should be tested for leaks and if found defective must be rejected. It has a total length of 55 to 56 cm., an outside diameter of 10 to 11 mm., and an inside diameter of 8.25 mm. (± 0.25 mm.); it ends in a capillary 3 to 3.5 cm. long which has an outside diameter of 3.25 mm. (± 0.25 mm.) and a lumen of 1.5 to 2 mm. A right-angle side-arm inlet is fused on 1.5 cm. below its mouth; this side arm has an outside diameter of 5 to 5.5 mm. and an inside diameter of 1.5 to 2 mm. The arm which is sealed to the combustion tube is 4 to 4.5 cm. long, and the other, attached glass-to-glass with a 3-cm. length of aged rubber tubing to the outlet of the U-tube, measures 2.5 to 3 cm. in length.

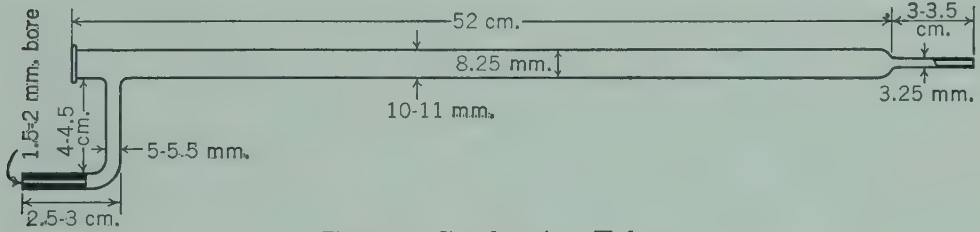


FIG. 30. Combustion Tube.

COMBUSTION-TUBE FILLINGS

"Simple" Filling. This type of filling is for substances *not* containing nitrogen. It consists of a platinum wire gauze, silver wool, copper oxide, followed by another layer of silver wool. Since the lead peroxide, with its objectionable adsorption and absorption properties, is eliminated in this filling, excellent results, particularly hydrogen values, are obtained. No heating mortar is required.

"Simple Band" Filling. This filling is designed for compounds not containing nitrogen but possessing highly condensed ring systems (phenanthrene, chrysene, sterol type of compounds, etc.), particularly those containing an *angular* methyl group, which upon pyrolysis comes off in the form of methane, which often is likely to escape complete combustion by a mere copper oxide or copper oxide-lead chromate filling.[106a, 113] The filling consists of five layers, each 3.5 cm. long.

Three layers, of copper oxide, are alternated with two layers of 30% platinized asbestos, as shown in Fig. 31. In addition, this filling has the usual layer of silver wool at the capillary end and a platinum wire gauze at the beginning of the filling. Also for this filling no heating mortar is required.

"Combination" Filling.[95] This type of combustion-tube filling is necessary for substances containing nitrogen. It is similar to the above *simple* combustion-tube filling, except that it contains a layer of lead peroxide after the oxidation filling which consists of the platinum wire gauze, copper oxide, and silver wool. The lead peroxide requires special heat treatment; therefore the heating mortar is necessary.

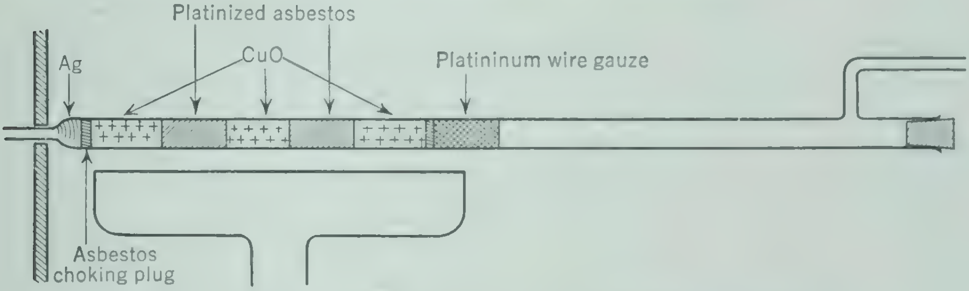


Fig. 31. Combustion Tube with the "Simple Band" Filling.

"Combination Band" Filling. This filling combines the features of the *simple band* filling with the *combination* combustion-tube filling. Thus it really becomes the filling of universal applicability, since any organic substance, those containing nitrogen as well as those giving low carbon values in insufficiently catalyzed fillings, can be analyzed satisfactorily. The filling consists of the alternating three layers of copper oxide and two layers of platinized asbestos, followed by silver wool and lead peroxide. A platinum wire gauze is placed at the beginning of the filling. The lead peroxide requires the inclusion of a heating mortar in the apparative set-up.

Filling of the Combustion Tube. (Since the combustion train is needed to test the resistance of the combustion-tube filling to the pressure of oxygen while the tube is being filled, in order to avoid either insufficient or excessive pressure, the filling is done after all other parts of the apparatus are set up and connected.) After the combustion tube has been cleaned and dried as described on p. 83, the procedure of filling it with the *combination filling*, the one most widely used, is carried out as follows:

Silver wool or silver wire wound in spiral form is introduced into the combustion tube and placed against the capillary ending with a wooden rod to form a loose layer 1 cm. long. This is followed by a 5- to 6-mm. compact layer of asbestos, the so-called choking plug. The purpose of this asbestos plug is to establish the correct resistance to the pressure of oxygen—an important factor, because excess resistance will slow down the flow of oxygen to below the required velocity; insufficient resistance, however, has the reverse effect. Ideal combustion conditions require a flow of 4 to 5 ml. of oxygen per minute through the system. The resistance offered by the choking plug is tested by attaching the combustion tube to the apparatus as shown in Fig. 25, but omitting the absorption tubes. To take into account the expansion of the asbestos upon heating, the liquid in the heating mortar is brought to boiling.

After the safety tube has been attached to the combustion tube, the side arm of the Mariotte flask is lowered to a horizontal position, the pressure regulator adjusted to 3 cm. below the level of the liquid, and the amount of water displaced in the Mariotte flask by the oxygen measured with a graduate cylinder. The choking plug has the correct resistance if 7 to 8 ml. of oxygen passes through the system per minute under the above conditions. If insufficient or excessive pressure is observed, it must be remedied by either adding more asbestos or removing part of the choking plug already in the combustion tube. When the correct resistance has been attained the combustion tube is removed from the apparatus and its inside wiped with a cotton wad wound around a stiff iron wire to remove asbestos fibers adhering to the side wall. Next, a 3.5- to 4-cm. layer of lead peroxide is added and the combustion tube gently tapped to avoid the formation of air pockets between the granules of the reagent. The combustion tube is again wiped with a cotton wad to remove the powder of lead peroxide from its side walls. This cleaning is repeated three or four times, and then another asbestos plug 1 to 2 mm. long is gently pressed against the lead peroxide to hold it in place. The combustion tube is again attached to the apparatus and its resistance tested; a flow of 5 to 6 ml. of oxygen per minute through the system under the conditions stated above should be obtained. The combustion tube is wiped clean of asbestos fibers, and enough silver wool or spirals of silver wire are added to form a layer 3.5 to 5 cm. long which, depending upon the measurements of the set-up, are distributed as follows: 1 to 2 cm. are to be within the heating mortar; 0.5 to 1 cm. to cover the space between the mortar and the electric furnace or long burner; and 1 cm. to extend into the heating unit. An asbestos plug 1 to 2 mm. long is placed against the silver wool and then copper oxide is added. The exact length of this filling depends on the measurements of the electric furnace or long burner, but enough oxidizing agent is added, a small portion at a time and with repeated tapping, to form a compact layer approximately 14 cm. long, but still 2 to 2.5 cm. from the end of the heating unit. The combustion tube is again wiped clean and another asbestos plug 1 to 2 mm. long is lightly pressed against the copper oxide. A platinum wire gauze of 60 to 80 mesh and measuring 3 by 5 cm., rolled around some silver wire to form a cylinder 7 mm. in diameter and 3 cm. long, is pushed into the combustion tube with a glass rod; the platinum gauze should extend about 1 cm. beyond the heating unit. (The correct distribution of the filling of the combustion tube is shown is Fig. 32.) Finally, the combustion tube is again attached to the apparatus and its resistance to the flow of oxygen is tested once more. The additional filling should have reduced the flow of oxygen to 3 to 4 ml. a minute. To obtain additional pressure the jacket of the pressure regulator is depressed to give a head of 4 to 5 cm. During these tests, which are made to establish the correct pressure in the system, the side arm of the Mariotte flask should remain in a horizontal position; changes in the pressure should be made by the pressure regulator, and the side arm of the Mariotte flask is to be used only for the final or exact regulation, so that it is never more than a few degrees off its horizontal level.

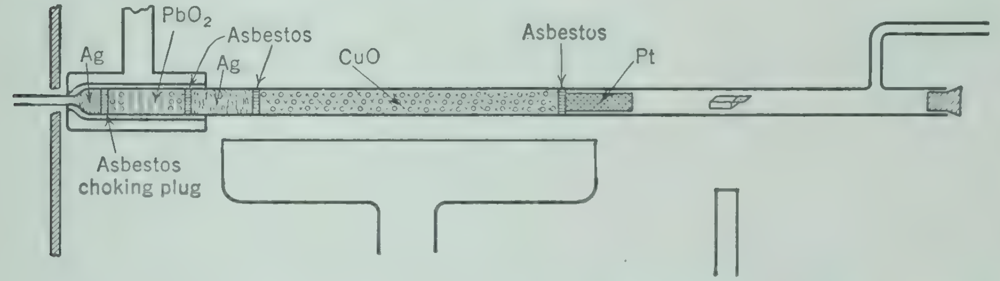


FIG. 32. Combustion Tube with the "Combination" Filling.

Conditioning of the Combustion Tube. A freshly filled combustion tube must be heated for a period of time before it can be used for analyses. This preheating is carried out by attaching the combustion tube to the apparatus, but leaving its capillary end open and adjusting the pressure regulator to a delivery of 3 to 4 ml. of oxygen per minute. The combustion tube is heated for at least 8 hours, or preferably over night, with all the heating units showing the normal combustion temperature of 700° C. (±25° C). This preheating process, however, causes an abnormal dehydration of the lead peroxide and consequently the first few analyses usually give a low hydrogen content. To restore the moisture equilibrium, the combustion of a few unweighed samples (approximately 10 mg.) is advised.

When not in use, the combustion tube is closed by attaching the safety tube of the Mariotte flask to its capillary end; it is always kept under pressure of oxygen to prevent atmospheric vapors from entering the system. A combustion tube protected in this manner will remain in good condition even after prolonged idleness and need only be heated for several hours before a combustion is carried out.

In the *simple* combustion-tube filling the filling operation and the tests for the gas-flow resistance remain the same, except that the lead peroxide is omitted. No heating mortar is required; however, the capillary outlet of the combustion tube must be adequately insulated against overheating.

A similar procedure is followed for the *simple band* filling. Also here the lead peroxide and heating mortar are omitted. Instead of a single layer of copper oxide, however, three layers of copper oxide, each separated from the other by a layer of 30% platinized asbestos, as shown in Fig. 31, are used; each layer is 3.5 cm. long. Although the platinized asbestos layers should be fairly compact, they must not be pressed together too tightly, for otherwise excessive resistance to the flow of oxygen would result. Thus it is advisable to test the flow of oxygen through the system after the introduction of each layer of platinized asbestos and make certain that the pressure obtained fulfills the requirements stated in the paragraphs describing the filling of the combustion tube (p. 108).

The *combination band* filling is identical with the *combination* filling, i.e., retention of the lead peroxide and the heating mortar, but with replacement of the single copper oxide layer by alternate 3.5-cm. layers of copper oxide and platinized asbestos, as described for the *simple band* combustion-tube filling.

The combustion tube always should be adequately protected from the flames of the long burner by a fine-mesh wire gauze which is somewhat longer than the burner, and from the flame of the Tirrill or the Bunsen burner by a wire gauze about 5 cm. long. If an electric furnace is employed the long wire gauze is omitted; no wire gauzes are needed for a quartz combustion tube.

Absorption Tubes (Fig. 33). Because the accuracy of a carbon and hydrogen determination depends considerably upon the constant weight attainable by the absorption tubes, their construction and also their measurements, especially those of the capillary constrictions, are of utmost importance. The water absorption tube is approximately 17 cm. long over all. The filling chamber has a diameter of 8 to 9 mm. and is 8 to 9 cm. long; it is separated from the adjoining air chamber by a thin glass wall having an aperture of 0.20 to 0.25 mm. The absorption tube ends in a capillary tubing of about 3-cm. length and 3.3- to 3.5-mm. outside diameter, or the same diameter as specified for the capillary ending of the combustion tube. This capillary tubing of the absorption tube has two constrictions, each being 5 mm. long and having an inner diameter of 0.20 to 0.25 mm.; the two constrictions are separated from each other by an air chamber 3 mm. long and 2 to 2.5 mm. in inner diameter. This design of the capillary ends is intended to reduce the diffusion with atmospheric vapors, especially moisture, thus contributing greatly to the constancy of weight attainable by the absorption tube. The so-called head or open part of the absorption tube has a ground-glass joint provided with a hollow ground-glass stopper which has an aperture 0.2 to 0.25 mm. in diameter at its curved base; its opposite end is drawn out to a capillary tubing of the same construction and measurements as the end of the absorption tube.

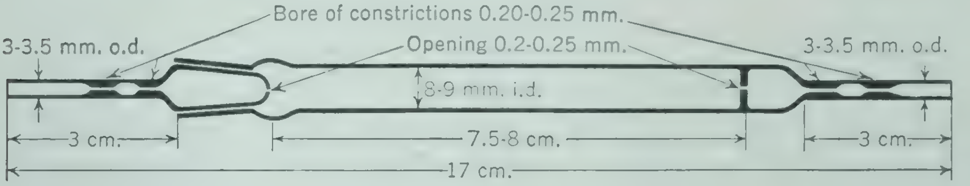


FIG. 33. Absorption Tube.

The carbon dioxide absorption tube is identical in construction and measurements with the water absorption tube.

Water Absorption Tube. The water absorption tube is cleaned by washing with distilled water and rinsing with alcohol afterwards. It is dried by being placed first on the suction pump, next in a drying oven kept at 120° C. This is followed by another drying on the suction pump to remove the alcohol vapors completely. The tube is filled by placing a 3- to 4-mm. layer of pliable glass wool against the end of the filling chamber, which is intended to protect the aperture of the glass wall from particles of Anhydrone. A 1.5- to 2-cm. layer of coarse Anhydrone (flakes 3 to 5 mm. long) is then introduced, followed by another 3- to 4-mm. layer of loosely packed glass wool. The rest of the absorption tube, to within 5 mm. of the ground-glass joint, is filled with fine but not powdered Anhydrone. Another wad of glass wool, filling the remaining space in the absorption tube, completes the filling. The ground-glass stopper is warmed outside the flame of a microburner and a little Krönig's glass cement is applied to the warm surface of the stopper, which is then quickly inserted and rotated in the ground-glass joint of the absorption tube; a transparent seal should be obtained. If insufficient cement has been applied, the ground-glass joint is warmed outside the flame with constant turning until the cement softens and the stopper can be removed. More cement is then applied and the sealing repeated. Excess cement must also be avoided, lest the aperture at the base of the stopper should become clogged when the stopper is placed in the ground-glass joint. The excess cement collecting on the rim of the ground-glass joint is removed with a cotton tuft dipped in benzene.

Carbon Dioxide Absorption Tube. After the tube has been cleaned as described above, a wad of pliable glass wool, followed by a 2.5- to 3-cm. layer of fine Anhydrone, is introduced. Another small wad of glass wool is then introduced and the rest of the tube is filled with Ascarite within 5 mm. of the ground-glass joint. A third layer of glass wool is added and the absorption tube sealed as described above.

Before the absorption tubes are used for an analysis, their resistance to the flow of oxygen or air must be tested. They are attached to each other head-to-head with a thick-walled rubber tubing 3 cm. long. Another such rubber tubing is placed on the free end of the water absorption tube, and the absorption tubes thus connected are attached to the capillary ending of the Ascarite-Anhydrone tube of the aspirator; the free end of the carbon dioxide absorption tube is connected to the safety tube of the Mariotte flask. Then the side arm of the Mariotte flask is adjusted to a horizontal position, and under this condition a minimum flow of 3 ml. of air per minute should be obtained. Fifty milliliters of air is drawn through the system in this manner and then, if the filling permits the passage of air at the above-mentioned velocity, the tubes are ready for use; otherwise it must be determined which one of the tubes is filled too tightly by attaching them separately. The faulty absorption tube must be refilled because a greater resistance than stated above may lead to difficulties soon after a few analyses, owing to the increase of the resistance as the filling of the absorption tubes is gradually used up.

Counterpoising the Absorption Tubes. According to F. Pregl [106b] the absorption tubes may be counterpoised with open tare bottles containing lead shot, and to distinguish them from each other they are provided with numbers or letters, for example, *H* for the Anhydrone tube and *C* for the Ascarite tube. This form of counterpoising has been found entirely satisfactory when running analyses on days with slight temperature and humidity changes. Since lead shot tends to take up moisture—10 grams takes up as much as 6 micrograms per 1% increase in humidity,[128] a tare bottle with a ground-glass stopper should be employed. Glass beads may be substituted for the lead shot, but then much larger tare bottles are required. Another method of counterpoising the absorption tubes involves the use of a so-called tare absorption tube.[52, 93] Three absorption tubes of approximately equal weight are selected. One serves as the water absorption tube and the second as the carbon dioxide absorption tube. Both are filled according to the directions given above but, for the time being, are not sealed. The third absorption tube serves as the tare for the two others and is filled half with Ascarite and half with Anhydrone. The weight of the three tubes is adjusted while they are still open by inserting or removing appropriate amounts of absorption reagents until their weights are within 0.5 gram of each other. Then they are sealed as usual. The tare absorption tube undergoes identical wiping and weighing treatment and is attached to the other two during the combustion in such a manner that the Ascarite layer is adjacent to the carbon dioxide tube and the layer of Anhydrone faces the safety tube of the Mariotte flask. In the actual weighing of the absorption tubes, the tare absorption tube is placed on the hooks of the right-hand pan of the balance. Thus not only does the tare absorption tube serve as a control [41f] but also, since it undergoes identical treatment with the absorption tubes, any climatic influences such as changes in temperature, atmospheric pressure, and humidity are automatically taken care of.

To prevent the absorption of water or carbon dioxide by the absorption tubes when they are not in use, so-called *protection caps*—thick-walled rubber tubing stoppered at one end with a fire-polished glass rod, or rubber stoppers having a bore that extends to only half the length of the stopper—are placed over the capillary constrictions.

Drying Tube and Safety Tube (Fig. 25, II, X). The purpose of these tubes is to absorb moisture that may emanate from the pressure regulator or the Mariotte flask. The tubes are identical in size and construction and are 13 cm. long and 10 to 12 mm. in diameter. Each tube is open at one end and has an air chamber at the other which terminates in a capillary bent at a right angle which is 3 to 3.5 cm. long and has an outer diameter of 4 mm. The tube is filled with Anhydrone, and a fairly tight layer of pliable glass wool is placed before and after the filling. The open end is closed with a rubber stopper through which a capillary bent at a right angle is inserted. A rubber tubing about 30 cm. long connects the drying tube to the preheater and another of approximately 60-cm. length connects the safety tube to the Mariotte flask.

Mariotte Flask (Fig. 25, XI). The purpose of the Mariotte flask is to measure the volume of the oxygen passing through the system and, is to measure the volume of the oxygen used in the combustion and, furthermore, to supply sufficient suction to overcome the resistance offered by the absorption tubes, so that approximately atmospheric pressure prevails at the rubber connections between the capillary end of the combustion tube and the water absorption tube and between the latter and the carbon dioxide absorption tube. Maintenance of atmospheric pressure conditions at these points is quite important in order to minimize either loss of combustion gases in case of excess pressure, or assimilation of moisture from the air in case of reduced pressure. The Mariotte flask may be of 1- or 2-liter capacity, the larger size being preferable because it permits the carrying out of at least eight determinations without refilling. A glass tube or side arm is fixed into the lower tubulure of the flask by means of a perforated cork stopper, an arrangement which permits easy adjustment of the side arm. The side arm should have a length equal to the height of the Mariotte flask (23 to 25 cm. for a 2-liter flask); it has an outside diameter of about 5 mm. and a bore of 3 mm. It is bent at a right angle about 5 cm. from the end which is inserted into the cork. The other end is bent vertical to the horizontal side arm and drawn out to a capillary tip of 1- to 1.5-mm. bore and 2.5- to 3-cm. length. The neck of the Mariotte flask is provided with a one-hole rubber stopper; a glass tube of about 3-mm. bore, bent twice at a right angle and carrying a three-way stopcock [68] between the bends, is inserted through the rubber stopper and extends almost to the bottom of the flask. The Mariotte flask is placed high enough on a suitable stand so that a 250-ml. graduate cylinder, in which the actual displacement of water by the oxygen during the combustion is measured, can conveniently be placed under the horizontal side arm.

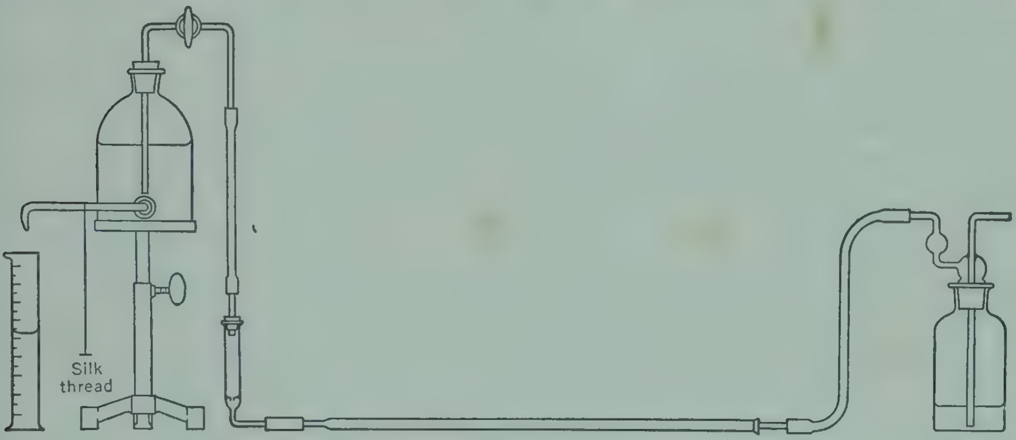


Fig. 34. Aspirator.

Aspirator [97] (Fig. 34). To replace the oxygen in the absorption tubes with air after the combustion and to cool them quickly to the temperature of the balance room, which is necessary, a separate unit, *the aspirator*, is employed. It is completely separated from the combustion train and, because of its simplicity and mobility, can be quickly set up in any convenient place in the balance room or near the balance, a circumstance which greatly aids in obtaining the much-desired constancy of weight of the absorption tubes. This aspirator consists of a wash bottle of 250-ml. capacity, a combustion tube—which may be an old, though clean, combustion tube—a drying tube, and a 1-liter Mariotte flask. The wash bottle contains about 70 ml. of concentrated sulfuric acid. A combustion tube, cut to a length of 25 cm., but with the capillary end in perfect condition, and its mouth provided with a perforated, tight-fitting rubber stopper into which a capillary glass tubing is inserted, is connected with a good rubber tubing to the exhaust of the wash bottle. The filling of the aspirator tube consists of the following: a plug of pliable glass wool approximately 5 mm. long is placed against the capillary ending; then a 10-cm. layer of Anhydrone is introduced, followed by a wad of glass wool and a 10-cm. layer of Ascarite; a third plug of glass wool is placed against the Ascarite and the rest of the tube is filled with Anhydrone, which is followed by another layer of glass wool. This filling is designed to remove carbon dioxide and moisture from the air which is passed through the absorption tubes after the combustion. The Mariotte flask, placed on a stand as illustrated, has the same side-arm arrangement and stopcock tubing at the top as the one of the combustion train. Rubber tubing about 50 cm. long leads from the top of the Mariotte flask to the safety tube, which is filled with Anhydrone. The right-angle capillary tube of this safety tube carries a thick-walled rubber connection about 3 cm. long with which it is attached to the carbon dioxide absorption tube when in use, or to the capillary ending of the aspirator tube when not in use. A silk thread is attached to the delivery tube of the Mariotte flask to test for electric charges on the absorption tubes after they have been wiped.

Miscellaneous

Flannel. A piece of flannel about 10 cm. square is kept in a Petri dish. It is moistened before use, but should not be wet; excess moisture must be removed by squeezing the flannel and pressing it between the folds of a towel.

Chamois. Several pieces of chamois about 10 cm. square are needed and are kept in a Petri dish. They should be of a good grade of soft leather and should be freed of fat and other impurities when new, and whenever necessary thereafter, by washing them thoroughly with soap and warm water to which a little ammonium hydroxide has been added.

They are rinsed thoroughly and dried at room temperature. To prevent the accumulation of electric charges on the absorption tubes when wiping them on very cold or dry days, it is recommended that a slightly moist flannel be placed on the bottom of the dish in which the chamois are kept.

Rubber Tubing. The rubber tubing from the oxygen tank to the pressure regulator and thence to the preheater should be of a good grade and about 7 mm. in outside and 4.5 to 5 mm. in inside diameter. It is not necessary to subject it to the aging process if a preheater is employed in the combustion train, although impurities present in the bore such as powder or talcum should be removed.

Rubber Connections. The rubber connections between the preheater and the bubble counter and between the U-tube and the side-arm inlet of the combustion tube must be of seamless and aged rubber tubing of 3.5-, to 4-mm. bore and the thickness of the wall should be at least 1 mm. The rubber connections employed for the absorption tubes are of thick-walled, seamless impregnated rubber tubing of about 1-cm. outside diameter and 2-mm. bore (± 0.25 mm.). The process of impregnation is carried out by cutting vacuum rubber tubing of the above dimensions into pieces 3 cm. long, placing them in a 500-ml. round-bottomed flask containing 150 to 200 ml. of pure vaseline or paraffin wax, and heating them on the water bath for about twenty minutes. While still warm the flask is then evacuated on the suction pump, and as soon as the contents have ceased to foam the vacuum is broken to admit the air which forces the paraffin or vaseline into the pores of the rubber tubing. This procedure of boiling and evacuation is repeated several times until at a reduced pressure of 10 to 15 mm. no further rising of bubbles is observed. The paraffin or vaseline is permitted to drain off while still warm and the rubber tubings are wiped clean on the outside with a clean cloth and the bore with tufts of cotton moistened with a little benzene and finally with dry cotton. The impregnated and cleaned rubber tubings are stored in a desiccator over Anhydrone.

The two rubber connections for the absorption tubes are marked I and II, so that their respective positions are always maintained. Rubber connection I is placed at the end of the water absorption tube and II between the water absorption tube and the carbon dioxide absorption tube. In addition, an arrow may be painted on the rubber connections to indicate the direction of the gases passing through them; this assures identical placement each time they are attached. Correct placement is especially important for rubber connection I, one end of which becomes prematurely decomposed by its exposure to the warm capillary end of the combustion tube.

Stopper for the Combustion Tube. Although not free of objectionable properties, such as porosity and hygroscopicity, a smooth, tight-fitting cork is best suited for this purpose. Rubber stoppers meet with disfavor because particles of rubber break off after short usage and may be carried to the area of the combustion when the platinum boat or foil is introduced.

Assembling the Combustion Train

A table of convenient height and suitable length (1 meter high and 2 meters long) which allows easy access to, and complete survey of, all parts of the apparatus, should be chosen as the basis of the set-up. Depending upon its size and the space available, the oxygen tank is placed close enough to the combustion train so that a 30- to 40-cm. length of rubber tubing, attached to the outlet of the reduction valve, is sufficient to connect it to the pressure regulator.

(With the exception of the combustion tube, for the filling of which a completed set-up is required, all other parts of the apparatus should be ready for attachment. Such glass parts as the pressure regulator, drying and safety tubes, preheater, bubble counter, and U-tube are cleaned, dried, and filled as previously described.)

The pressure regulator, following the oxygen tank in the order of the set-up, is filled to two-thirds of its capacity with dilute sulfuric acid (1 : 1). The vertical end of the intake tube of the pressure regulator is moistened with a trace of glycerin, and then the rubber tubing leading from the oxygen tank is placed over it. The rubber tubing should have a bore of 3.5 to 4 mm., or an inside diameter smaller by 0.5 to 1 mm. than the outside diameter of the glass tube, to insure a gas-tight connection. Rubber tubing having a bore considerably smaller than stated is unsuitable, because as the result of excessive stretching it soon develops cracks at the joints and, consequently, leaks. The outlet tube of the pressure regulator is moistened with a trace of glycerin and inserted in the perforated rubber stopper of the drying tube. A rubber tubing approximately 25 cm. long is attached to the right-angle capillary tube of the drying tube and connected to the inlet of the preheater. To insure tight connections and a leak-proof system all rubber tubings should be placed 1.5 to 2 cm. beyond the end of the glass tubing to which they are attached.

The preheater is fastened to a suitable metal stand in the following manner: A cylindrical cork, cut open parallel to its axis and having a bore of 6 to 7 mm., is placed just below the combustion tube of the preheater which is attached to the stand with a suitable clamp. The spiral, fastened to the preheater with two wire springs, is immersed in a conical beaker three-fourths filled with water and held at the proper height by a ring clamp. Another ring clamp, placed over the combustion tube at about the height of the inlet tube, serves as a support for an asbestos shield or Transite plate approximately 15 by 15 cm. with an opening in the center so that it can be placed over the vertical combustion tube. This shield protects the rubber tubings at both ends from the effects of the heat from the electric heating unit or the gas burner. If a Tirrill or Bunsen burner is used, it is provided with a wing top to effect an efficient heat distribution over a larger part of the combustion tube. The height of the preheater is so adjusted that its outlet tube is on the same level as the inlet tube of the bubble counter.

The bubble counter and U-tube is either suspended from a stand with a wire stirrup or loosely held in place or supported by a clamp attached to a small stand. It is held at such a height that the outlet tube is level with the side arm of the combustion tube. The inlet tube of the bubble counter is connected glass-to-glass to the preheater and the U-tube glass-to-glass to the side arm of the combustion tube, as shown in Fig. 25. The rubber tubings with which these connections are made must be of best-quality impregnated material and are about 3 cm. long, of 6-mm. external and 4-mm. internal diameter. They should be moistened as little as possible when they are attached, and they have to be pushed over the glass tubings as a tight-fitting glove is drawn over the fingers. When the bubble counter or the combustion tube is removed it is better to cut off these rubber connections, because after some time they will adhere to the surface of the glass. Other means of removal than cutting will incur considerable risk of breakage. By attaching the bubble counter and U-tube to the apparatus glass-to-glass, the possibility of organic matter being taken up by the oxygen at these points is practically eliminated. And since combustible impurities present in the oxygen either originally or because of the contact of the oxygen with the two longer rubber tubings before and after the pressure regulator, are combusted by the preheater to carbon dioxide and water and subsequently absorbed by the filling of the U-tube, these rubber tubings, otherwise a potential source of trouble, should no longer be the cause of high carbon and hydrogen values.

The long gas burner, the combustion stand, and the heating mortar are assembled next. The height of the heating mortar is adjusted so that the combustion tube fits into the central passage but still rests on the V-shaped notches of the stand. A strip of asbestos paper, 6 cm. long and 1.5 cm. wide, is placed in this passage under the combustion tube to give it some support without, however, obscuring the combustion tube, visible through the glass heating mortar. A disk of heavy asbestos paper, or several to bring it to 2- to 3-mm. thickness, is cut to conform to the diameter of the glass jacket to fit between the heating mortar and the combustion stand. The other end of the heating mortar which faces the absorption tubes is similarly provided with a disk of asbestos paper and then with an asbestos shield or Transite plate about 30 cm. high and 20 cm. wide, having an opening at the proper height to permit the passage of the capillary ending of the combustion tube. This shield is necessary to protect the absorption tubes from the heat emanating from the heating unit. It should be tied to the heating mortar with a pliable wire to keep it firmly in place. The long gas burner is placed under the stand and 1.5 cm. away from the heating mortar. Its height is so adjusted that the wire gauze of the combustion tube is covered by its flames, which should be of sufficient intensity to heat the wire gauze to a dull red.

If an electric furnace is employed [3, 55, 84, 117] its temperature must be adjusted to 700° C. (± 25°) by means of a suitable resistance; furthermore, it is necessary to test the heating element of the furnace at both ends as well as in the middle with a pyrometer for its uniformity in temperature (± 25° C.). A furnace which shows greater variations in temperature is unsuited for service. The electric furnace and the heating mortar are joined to each other as closely as possible. For the sake of greater stability it is advisable to fasten the one to the other with flexible wire.

With the Mariotte flask assembled and placed after the combustion train proper, as shown in the illustration, and the combustion tube filled and attached, the set-up of the carbon and hydrogen apparatus is essentially completed. The next step is to test the system for possible leaks, because it is quite obvious that leakages in the combustion train must be eliminated, first in order to avoid loss of combustion gases and second because only a gas-tight system will give the correct pressure conditions so essential for a satisfactory determination. The principal sources of leaks are the various rubber connections and are due either to poor-grade, very porous rubber tubings, or to tubings of improper diameter, for if the diameter is too large, the connection will not be tight enough to withstand the pressure within the system, and if too narrow, the tubing will be unduly stretched, resulting in excessive porosity. However, it is also possible that some glass part of the apparatus may have a small crack which might be responsible for the leak and, therefore, a thorough inspection of the entire system is necessary.

The apparatus is tested for leaks as follows: First, the pressure regulator is depressed to give a head of about 5 cm. and the capillary end of the combustion tube is closed with a thick-walled, tight-fitting rubber connection stoppered with a glass rod. Then the oxygen is turned on and the flow so regulated that about one bubble per second overflows from the jacket in the pressure regulator. If the level of sealing liquid inside the jacket remains the same and if at the same time no bubbles rise in the bubble counter, the apparatus can be considered air-tight and free of leaks. If, however, the meniscus of the sealing liquid in the jacket of the pressure regulator rises and if at the same time bubbles appear in the bubble counter, there might be not only one but several leaks. Rising of bubbles in the bubble counter always indicates a leak in the combustion train. Rise of the meniscus of the sealing liquid in the jacket of the pressure regulator, with absence of bubbles in the bubble counter, indicates a leak in the purification train between the pressure regulator and the bubble counter. The leaks may be detected by applying a little water to the connections under suspicion. In the event of a large leak, the faulty rubber connection must be replaced by a better one; if it is only a slow leak, that is, if only one bubble in ten or fifteen seconds passes into the jacket of the pressure regulator, these connections may be made leak-proof by painting them with a little shellac or collodion. During this operation and while the material is allowed to dry, it is necessary, of course, to reduce the pressure in the system to approximately atmospheric. After the painted connections have dried and all leaks have been eliminated, the apparatus, with the combustion tube attached, is heated for a period of at least eight hours or preferably over night. The exposed part of the combustion tube is also heated with a Tirrill or Bunsen burner for about thirty minutes, beginning from a safe distance from the cork stopper (about 5 cm.) up to the long burner or electric furnace. Finally, a blank analysis is carried out as described on p. 126, and if the increase in the weight of the absorption tubes is within tolerable limits—0.04 mg. for the water absorption tube and 0.02 mg. for the carbon dioxide absorption tube—then the apparatus is ready for use.

Reagents

Anhydrone (Magnesium Perchlorate).[7] It is sifted to remove the powder and separated into coarse and fine particles, the former representing flakes several millimeters long, and the latter being 1 mm. square or less. The two sizes are saturated with carbon dioxide and stored separately in wide-mouth ground-glass stoppered reagent bottles.

Ascarite (Sodium Hydroxide on Asbestos).[131] This commercial reagent can be used without further preparation; in filling the carbon dioxide absorption tube, however, larger pieces of asbestos should be avoided. It is stored in a wide-mouth ground-glass stoppered reagent bottle.

Asbestos. Gooch crucible asbestos is used for the asbestos plugs in the combustion tube. It is ignited in a porcelain crucible to red heat in an electric furnace for thirty minutes and is stored in a wide-mouth ground-glass stoppered reagent bottle. If an electric furnace is not available, the asbestos may also be ignited just before being used by holding small amounts with the platinum-tipped forceps in the slightly hissing, non-luminous flame of a gas burner.

Copper Oxide (Cupric Oxide).[7, 120] Copper oxide is crushed to pieces of 3- to 4-mm. length; particles smaller than 20 mesh are sifted out. The accepted portion is ignited in a porcelain crucible in an electric furnace at 800 to 900° C. for about thirty minutes with occasional stirring. If an electric furnace is not available, the crucible is placed in a larger one which rests on the heavy asbestos shield provided with an opening large enough so that the larger crucible can be heated effectively with a hot flame. The copper oxide is ignited with a strong flame for about one hour with occasional stirring.

Pliable Glass Wool (Corning Brand Fibre Glass No. 008).

Lead Peroxide (Lead Dioxide, Lead Superoxide, PbO_2).[106a, 113] Because only high-grade lead peroxide will give satisfactory results, it is necessary to devote considerable care to the preparation of this reagent or, if obtained from a commercial source, to test its purity. Commercial lead peroxide is purified and prepared as follows: About 100 grams of lead peroxide is digested with concentrated nitric acid in an evaporating dish on the steam bath for about two hours with occasional stirring. After standing for one hour the nitric acid is decanted and the product is washed with distilled water until the test of the wash water with diphenylamine-sulfuric acid is negative. The material is then evaporated to complete dryness and cut into 1- to 1.5-mm cubes. These cubes are placed in a wide-mouth glass-stoppered reagent bottle, and by shaking with a rotary motion the cubes are rounded off and pellets of 1- to 1.5-mm. diameter are obtained. Pellets and powder are separated by sifting; the powder may be used over again by moistening it with distilled water to form a paste which is dried and cut into squares as before. The reagent is stored in a tight-stoppered, wide-mouth reagent bottle. A good grade of granular commercial peroxide [82, 120] should be dark brown in color and neutral to litmus paper. Small amounts of sodium silicate ("water glass") may be used as a binder.

Silver Wool.[3] No. 34 silver wire is wound loosely around a glass rod 2 to 3 mm. thick until a roll of silver wire 3 to 4 cm. long and 6 to 7 mm. in diameter has been obtained. Four to five grams of silver wire will yield several of these rolls, or sufficient for one combustion-tube filling. It is advisable to reduce the silver wire in an atmosphere of hydrogen at about 400° C. in a clean combustion tube and then to heat it once more at the same temperature in a current of oxygen. Previously used silver wire is regenerated in the same manner.

Platinum Gauze.[3] A piece of platinum wire gauze of 52 to 80 mesh, measuring 3 by 5 cm., is rolled, or fastened with a silver wire if necessary, to form a cylinder of 7-mm. diameter and 3-cm. length. To clean the platinum wire gauze it is boiled several times in dilute nitric acid (1 : 1), washed with distilled water, and ignited to white heat in the non-luminous flame of a Tirrill or Bunsen burner. A previously used platinum wire gauze is regenerated by boiling in dilute hydrochloric acid (1 : 2), dipping in distilled water to remove the hydrochloric acid, then boiling in dilute nitric acid (1 : 1) and igniting to white heat. To facilitate the handling of the platinum wire gauze when cleaning it, a small wire loop is attached to one end.

Platinized Asbestos (30%).[3] A weighed amount of platinum scrap (old boats, electrodes, unusable wire gauzes, etc.) is dissolved in aqua regia (1 part of nitric acid and 3 parts of hydrochloric acid by volume). A calculated amount of asbestos is added to the resulting solution and the mass evaporated to dryness on a steam bath. The residue is then transferred to a porcelain crucible and ignited in an electric furnace at a temperature of about 1000° C. Prolonged heating, at least ten hours, is necessary to free the platinized asbestos thus prepared from volatile matter.

p-Cymene.[34] If the boiling point of this substance is not within 175–178° C. it should be redistilled and only the fraction having the above boiling-point range should be used.

Krönig's Glass Cement. It is prepared by melting and mixing 1 part of white beeswax with 4 parts of rosin and casting it in sticks about 10 cm. long and 6 to 7 mm. in diameter which are stored in suitable test tubes.

Testing the Apparatus

Wiping and Weighing of the Absorption Tubes. Incorrect wiping and weighing of the absorption tubes may lead to weighing errors of sufficient magnitude to render the analysis worthless. It is therefore necessary to practice this procedure until constancy of weight of the absorption tubes can be reproduced. As the first step the capillary constrictions of the counterpoised absorption tubes are cleaned with a thin cotton wad twisted around the knurled end of the iron wire. The surface of the water absorption tube, while the tube is held in one hand with a folded clean and dry chamois at somewhat below the middle, is wiped with the folded moist flannel with a gentle rotary motion from the middle of the tube toward the capillary end, and then in an identical manner with a clean and dry chamois leather until the chamois glides smoothly over the surface of the absorption tube; three such wipings with the chamois are generally sufficient. The position of the water absorption tube is then reversed, the wiped part is held with a chamois, and the other half of the tube is wiped first with the moist flannel and then three times with the chamois. The water absorption tube, which is always wiped first, is placed on the rack provided for it, and the carbon dioxide absorption tube is wiped in an identical manner and also placed on the rack. The rack is placed next to the balance on an asbestos board or cardboard. The purpose of placing the absorption tubes next to the balance immediately after wiping is to allow them to come to equilibrium with the temperature prevailing near or within the balance case. While this equilibrium is attained, which requires from ten to fifteen minutes, the zero reading of the balance is determined, because approximately fifty-five minutes will elapse between the weighing before and after the combustion and the zero reading may change sufficiently during the interval to cause an appreciable error in the result if not taken into consideration.

The water absorption tube is placed on the hooks of the left-hand pan of the balance by means of the wire fork ten minutes after having been wiped. The doors of the balance are closed, and, to save time, the rider on the beam is placed in the notch corresponding to the weight of the absorption tube, so that one may proceed with the weighing of the tube immediately after the fifteen minutes since the wiping have elapsed. The water absorption tube is weighed within ±0.01 mg. if a microanalytical balance is used, or within ±1 deflection unit if an ordinary analytical balance is employed. The water absorption tube is then replaced by the carbon dioxide absorption tube, which is weighed immediately and with the same accuracy. The water absorption tube is then placed on the balance once more and weighed again; the result should check within ±0.01 mg. with the weight previously obtained. The carbon dioxide absorption tube is also weighed a second time in the same manner. These duplicate weighings may be dispensed with once sufficient experience in the procedure of wiping and weighing has been acquired.

Because the absorption tubes attain constancy of weight approximately ten minutes after wiping and retain this equilibrium for about thirty minutes, the weighing of both absorption tubes should be carried out within twenty to twenty-five minutes. It is important that they always be weighed in the same order, that is, the water absorption tube first and then the carbon dioxide absorption tube; furthermore, since the time schedule of weighing them must be strictly observed, timing of the weighings with the stop watch is recommended. Occasionally it seems rather difficult to obtain constancy of weight, even though all the conditions stated above have been complied with. This difficulty often can be traced to the accumulation of electrostatic charges on the absorption tubes, especially on dry or cold days. A test for the presence of such charges is performed by bringing the capillary end of the absorption tube near a small piece of tissue paper suspended from a silk thread, which, if an electrostatic charge is present, will be either attracted or repelled. To dissipate such charges the two ends of the absorption tube are touched simultaneously with a metal wire.

Blank Test on the Aspirator. Aside from the constancy of weight of the absorption tubes during a series of weighings it must be possible to reproduce their weight within the allowable limit of deviation (±0.01 mg. if a microanalytical balance is used, or within ±1 deflection unit if an ordinary analytical balance is employed) after they are wiped again. Such an experiment is carried out on the aspirator as follows: The absorption tubes after being weighed are connected glass-to-glass and head-to-head, that is, with the ground-glass stoppers facing each other,* with rubber connection II, so that the arrow which indicates the direction of the flow of the gases points toward the carbon dioxide tube. Rubber connection I is attached to the free end of the water absorption tube, with the arrow pointing toward the absorption tube, and then connected to the capillary end of the Anhydrone-Ascarite tube of the aspirator.† The free end of the carbon dioxide absorption tube is attached to the rubber connection of the safety tube of the Mariotte flask. Then the side arm of the Mariotte flask is lowered until a delivery of 8 to 10 ml. of water per minute is obtained. (The aspirator should be tested for tightness of the connections at this time by pinching the rubber tubing leading from the wash bottle to the aspirator tube, a manipulation which should stop the flow of water from the Mariotte flask; a loose connection will aspirate atmospheric vapors still containing moisture and carbon dioxide, and this will cause an appreciable increase in the weight of the absorption tubes.) Fifty milliliters of air is passed through the absorption tubes, after which the stopcock on top of the Mariotte flask is closed and the absorption tubes are disconnected from the aspirator. Rubber connections I and II are removed and placed in a Petri dish. Next, the capillary constrictions of the absorption tubes are cleaned with a tuft of cotton and then they are wiped and weighed in the same order and with the exact observance of the same time schedule as before. The result should check within ±0.01 to 0.02 mg. on a microanalytical balance and within ±1 to 2 deflection units on the ordinary analytical balance. If a greater deviation is observed, the experiment should be repeated until the proper technic of wiping and weighing the absorption tubes has been acquired.

---

* The absorption tubes are always connected head-to-head; disregard of this arrangement necessitates refilling the misplaced absorption tube. The combustion products are mainly absorbed at the entrance, and consequently, if reversed, some of the absorbed combustion products would be swept out or into the adjoining tube. Subsequent reversal to the correct position then produces the same effect, and hence it is impossible to restore the original equilibrium in the absorption tubes.

† Should the rubber tubings adhere too tightly to the absorption tubes they are lubricated with a trace of glycerin applied to a cotton tuft wound around the knurled end of the iron wire, which is then passed through the bore of the rubber connection. All excess glycerin is removed by wiping out the bore with a dry cotton wad. In general, rubber connection II requires lubrication about every fifth determination, and rubber connection I, owing to its proximity to the heating unit, about every second or third determination.

Blank Combustion Tests. The blank determination serves the purpose of giving assurance of a satisfactory condition of the set-up, particularly a new one, and furthermore is a means of detecting sources of errors or defects either inherent or developed during the use of the apparatus. It is performed on a heated combustion train, that is, the preheater, electric furnace or long burner, and the microburner under the heating mortar are lighted or turned on. The absorption tubes are wiped and weighed as described before and connected head-to-head with rubber connection II to form a glass-to-glass connection. Rubber connection I is attached to the free end of the water absorption tube, which is attached glass-to-glass to the capillary end of the combustion tube, and the free end of the carbon dioxide absorption tube is joined to the safety tube of the Mariotte flask.

The flow of oxygen through the system is standardized to the correct combustion conditions by adjusting the pressure regulator to a head of about 5 cm. and then lowering the side arm of the Mariotte flask to a horizontal position. As soon as bubbles rise outside the jacket of the pressure regulator, the stopcock on top of the Mariotte flask is opened. Water will begin to drip from the side arm; after a few seconds, or when the flow has become regular, it is measured in a 10-ml. graduate cylinder and timed with a stop watch. The correct pressure in the system, as measured by the displacement of water in the Mariotte flask, is reached when a discharge of 5 ml. of water per minute has been obtained. To increase the rate of flow of oxygen through the system, the jacket of the pressure regulator is depressed, or, if the difference is very slight, the side arm of the Mariotte flask is lowered; to decrease the pressure in the combustion train the head in the pressure regulator is reduced, or the side arm of the Mariotte flask raised. At no time, however, should the side arm be off more than a few degrees from its horizontal level; the pressure should be regulated principally by the pressure regulator and only the finer adjustment is made by manipulating the side arm of the Mariotte flask.

An additional means of calibrating the volume of oxygen passing through the system per minute is provided by the bubble counter by counting the number of bubbles rising from the aperture during a given time—10 seconds for instance—after the correct pressure has been established. Subsequent countings, taken at any time, either before a combustion is started or during the combustion itself, should then give assurance that pressure conditions are normal if the same count is obtained, or call attention to a fluctuation or possible disturbance in the event of a deviation. One hundred and fifty milliliters of oxygen is passed through the combustion train at the rate of 5 ml. per minute, after which the stopcock on top of the Mariotte flask is closed and the absorption tubes disconnected from the combustion train and immediately attached to the aspirator. About 50 ml. of air is drawn through the absorption tubes at a rate of 7 to 10 ml. per minute, and then they are detached from the aspirator and their capillary constrictions cleaned with a cotton tuft. They are wiped and weighed as before, and the increase in weight should be less than 0.04 mg. for the water absorption tube and 0.02 mg. for the carbon dioxide absorption tube if the apparatus is in satisfactory condition and the wiping and weighing have been performed properly.

The sources of error responsible for undue increases in weight of the absorption tubes are usually directly traceable to bad connections and leakages in the system, poor-quality rubber tubing, excessively lubricated rubber connections, or the filling of the combustion tube, U-tube, and also the absorption tubes. Since errors due to improper filling of the absorption tubes can be detected when air is passed through them on the aspirator, and the U-tube containing Ascarite and Anhydrone seldom is responsible for errors, unless the described arrangement of the filling has been entirely disregarded, only the rubber connections and the combustion tube remain to be tested. Of the rubber connections only the two used for the attachment of the absorption tubes may prove troublesome if not properly impregnated, or if excessively lubricated, or if the combustion is carried out under abnormal pressure conditions; the rubber connections on the combustion train should not give rise to any difficulties if a preheater is employed and all the connections between the preheater, bubble counter, and combustion tube are made glass-to-glass.

Whether the increase in weight in either one or both absorption tubes is due to the reagents in the purifying train (U-tube and the two Anhydrone tubes), or is traceable to the filling of the combustion tube, can be determined by running one blank on an unheated apparatus, with only the preheater lighted, and a second blank with the regular heating devices lighted or turned on. If the absorption tubes show undue increases in the first blank, the reagents of the purifying train can be held responsible and must be renewed; if, however, an increase is noted only upon heating the apparatus, the filling of the combustion tube is at fault. Of the reagents comprising the filling, the lead peroxide is likely to be the most troublesome, especially as far as the hydrogen values are concerned, owing to its characteristic of retaining water and then giving it off slowly over a prolonged period of time. Experimental observation has shown that the lead peroxide of a new combustion-tube filling gives off traces of water even after several hours of heating. The same observation can be made whenever the apparatus is heated after having been idle for some time. Heating out the apparatus for several hours while passing oxygen through at a rate of approximately 5 ml. per minute will, as a rule, remedy this fault. Should the high hydrogen values persist in spite of this treatment, it is best to refill the combustion tube with lead peroxide of a purer grade. A high-hydrogen blank may also be due to the hygroscopic properties of the rubber connections between the water absorption tube and the combustion tube, but this can readily be detected by placing them in a desiccator over a dehydrating agent several hours before the blank determination, although this rigorous dehydration should not be resorted to normally, because then a low hydrogen value would be the result. Should the carbon dioxide tube show a persistent gain in weight (more than 0.02 mg. during a blank determination), impure or improperly ignited copper oxide or platinized asbestos may be at fault.

Actual Analysis

After completion of a satisfactory blank determination, an analysis of a pure, known substance such as resorcinol, benzoic acid, or azobenzene is carried out. When correct results have been obtained the apparatus is ready for the analysis of unknown compounds or research substances. It is advisable, however, to continue to combust a few more compounds of different molecular structure, liquids as well as solids, containing also other elements, and varying in percentage composition of carbon and hydrogen, in order to study the behavior of different types of compounds upon combustion.

PREPARATION OF THE SAMPLE

Solids. The substance is weighed in a platinum boat which previously has been cleaned in the usual manner. Special weighing procedures (for hygroscopic substances, semi-solids, syrups, fats, waxes, etc.) are described in detail on p. 48.

Liquids. Liquid substances are weighed either in weighing pipets or, if the sample is not hygroscopic or volatile, in the platinum boat. The weighing pipet which contains a little potassium chlorate, is filled with the substance and weighed as described on p. 46. (Since the filling and weighing of the pipet are more time-consuming than the weighing of a solid substance, it is advisable to fill and weigh all the pipets for a series of determinations before the analyses are started.) Before being introduced into the combustion tube the pipet is again centrifuged, its tip broken off, and then placed in a previously cleaned platinum foil with the open end of the pipet facing the heating unit.

COMBUSTION

Attaching the Absorption Tubes. The weighed absorption tubes are connected head-to-head and glass-to-glass and then so attached to the combustion train that the free end of the water absorption tube is connected glass-to-glass to the capillary end of the combustion tube and the free end of the carbon dioxide absorption tube is joined to the safety tube of the Mariotte flask. The stopcock on top of the Mariotte flask remains closed for the time being.

Introduction of the Sample. During the introduction of the substance, the stopcock on top of the Mariotte flask remains closed to build up pressure in the system so that when the cork stopper is removed from the mouth of the combustion tube oxygen will escape rather than air be drawn into it. The sample is transferred to the combustion tube as follows: The stopper is removed and placed on the glass top of the dessicator; next, the metal block with the sample is raised to the mouth of the combustion tube, the platinum boat or foil is introduced with clean forceps and pushed with a clean glass rod within 5 cm. of the heating unit; then the combustion tube is closed tightly with the stopper.

Combustion of the Sample. The side arm of the Mariotte flask is lowered to a horizontal position, its stopcock opened, and the displacement of water by the oxygen passing through the system is measured and standardized to a flow of 5 ml. per minute, as described in the blank determination. The combustion is started about 5 cm. in front of the platinum boat or foil with a non-luminous slightly hissing flame 5 to 6 cm. high or high enough to extend about 2 cm. above the wire gauze of the combustion tube. The burner is gradually moved toward the substance and it should be noted whether it distils, sublimes, or chars. If the substance distils or sublimes, the ring of distillate or sublimate is slowly driven toward the heating unit. Substances which decompose with charring often require prolonged heating directly under the boat until all organic matter is burned off. The combustion is carried out with 75 ml. of oxygen passing through the system, as measured by its equivalent volume of water collected in a 250-ml. graduate cylinder, which, at the start of combustion, is placed under the side arm of the Mariotte flask. The combustion requires about fifteen minutes, divided approximately as follows: five minutes of heating to reach to sample, five minutes of heating directly under it, and five minutes to heat the intervening space from the sample to the heating unit. Explosive or difficultly combustible substances may require suitable modification of the combustion, but, nevertheless, the total time of fifteen minutes should not be greatly exceeded. Then the combustion is repeated, only more rapidly, again starting about 5 cm. in front of the platinum boat or foil; this second combustion requires about five minutes and is distributed by heating about one and one-half minutes to reach the platinum boat, two minutes of heating directly under it and one and one-half minutes to heat the combustion tube from the platinum boat to the heating unit. The movable gas burner is extinguished and the gaseous combustion products are swept into the absorption tubes for twenty minutes with 100 ml. of oxygen, thus requiring a total of about forty minutes for the entire combustion. During the forty minutes 200 ml. of oxygen will have passed through the combustion train and its equivalent volume of water collected in the graduate cylinder. Measuring the displacement of water by the oxygen in the Mariotte flask and comparing it with the time elapsed during the combustion affords a very accurate means of observing the smooth progress of a determination. Occasionally stoppages occur during the combustion; these are mainly due to condensation of water in the capillary constrictions of the water absorption tube, and the difficulty is remedied by applying the heated end of a metal rod or file to the affected constrictions.

After the sweeping out of the gaseous combustion products the stopcock of the Mariotte flask is closed, but its side arm is left undisturbed in the horizontal position, which eliminates the necessity of restandardizing the flow of oxygen at the beginning of the next determination. Once regulated, the pressure in the system will remain unchanged for an entire series of analyses.

Removal of the Absorption Tubes. The absorption tubes are removed from the combustion train and immediately attached to the aspirator without disconnecting them from each other; the free end of the water absorption tube is connected to the Ascarite-Anhydrone tube and the free end of the carbon dioxide absorption tube is attached to the safety tube of the Mariotte flask. Fifty milliliters of air is drawn through the system at a rate of 7 to 10 ml. per minute and measured by the displacement of water in the Mariotte flask. The absorption tubes are then removed from the aspirator and the capillary constrictions wiped with a clean cotton tuft. Finally, they are wiped, placed near the balance on a rack, and weighed after fifteen minutes as described on p. 123.

Rubber connections I and II are placed in a Petri dish, but on very humid days it is better to place them in a desiccator containing a weak desiccant such as calcium chloride.

It is advisable to time the individual steps of an analysis with a stop watch, especially when running a series of determinations. A time schedule, beginning with the wiping of the absorption tubes, is given below:

Time:

	Minutes
Wiping of the absorption tubes	5
Taking the zero reading of the balance	5
Waiting until absorption tubes become constant (15 minutes from time of wiping)	10
Weighing the water absorption tube	5
Weighing the carbon dioxide absorption tube	5
Reweighing both tubes	10
Attaching the absorption tubes to combustion train, introducing sample, standardizing the flow of oxygen	5
First combustion	15
Second combustion	5
Sweeping out the combustion products *	20
(During this time the sample for the next analysis is weighed)	
Removing the absorption tubes from the apparatus and attaching them to the aspirator and passing through 50 ml. of air	10
Wiping and weighing the absorption tubes	40
Total	135

---

* The sweeping-out period given above as twenty minutes should be extended to thirty minutes if water in an amount exceeding 2.5 mg. is collected and if a *combination band* combustion-tube filling has been used for this particular determination.

Calculation:

Percentage of Hydrogen:

Log of weight of water,
Plus log of factor (04875),
Plus negative log of weight of substance;
Antilog of total = percentage of hydrogen.

Percentage of Carbon:

Log of weight of carbon dioxide,
Plus log of factor (43600),
Plus negative log of weight of sample;
Antilog of total = percentage carbon.

Remarks

DRY COMBUSTION METHOD

The outstanding features of F. Pregl's [106a, 113] micromethod for the determination of carbon and hydrogen are:

(*a*) reduction of the size of the sample to a few milligrams,
(*b*) the saving of time, and
(*c*) perfect volume and pressure control.

Since the introduction of this method in organic elementary analysis in 1912 a large number of investigators [19, 25, 28, 33, 35b, 39, 41, 42, 75–78, 81, 88, 90–98, 115, 123, 124, 128, 132, 143–146] have studied and repeatedly reviewed this most important of all analytical methods in organic chemistry.

ACCURACY

The accuracy obtainable in this method depends upon the procedure employed and the skill and experience of the operator. The following claims have been made:

±0.1% by A. Friedrich; [41f]
±0.1–0.2% in F. Pregl's laboratory; [75]
±0.2% by A. Elek,[35a] D. F. Hayman,[51] and R. Roth; [112]
±0.3% general average.[113]

On the average, particularly when the apparatus is used by several non-expert operators, as in a laboratory course, it can be stated that the accuracy obtainable in the micro carbon and hydrogen determination is about the same as in the corresponding Liebig macro combustion method,[38] i.e.:

Allowable error (0.2-gram sample).
Carbon, 5 parts per thousand.
Hydrogen, 30 parts per thousand.

This statement appears also supported by F. W. Power[104] in calculations from statistical analysis data obtained from actual micro combustion analyses performed by several analysts in different laboratories. The above-given accuracy, however, must not be compared with atomic-weight determination for carbon by combustion analysis, in which a special apparatus and painstakingly purified substances in the amount of 1 to 3 grams were used.[9, 10, 37]

APPARATUS

Oxygen Tank. The previously used gasometers[106a, 113] have been replaced by an oxygen tank provided with suitable pressure-reduction valves. In regard to the oxygen used in the combustion it is necessary that it be free of organic impurities.[41b, 57] If a preheater is incorporated in the ante-combustion train, the purity and consequently the source of the oxygen, whether electrolytic oxygen or oxygen obtained by fraction distillation, is of little importance, because any combustible matter present in the oxygen stream is combusted to carbon dioxide and water, respectively, and consequently absorbed by the purifying train. The necessity of purifying both oxygen and air used in the combustion has been emphasized by a number of investigators.[18, 22, 97]

Preheater. The preheater of F. Böck and K. Beaucourt[18] fulfills this requirement admirably. It was improved by making the spiral tube detachable.[98] Thus a Pyrex or soft-glass spiral tube possessing a standard ground-glass joint can be used and only the vertical short combustion tube needs to be of quartz or Supremax glass. A horizontal short combustion tube[51] or a simple copper spiral[80] may be employed as a preheater, but care must be taken to protect the rubber attachments from heat. Combustion in oxygen only has been found suitable by many investigators.[41, 51, 65, 83, 97, 114, 126, 139, 147] Medicinal oxygen may be used without a preheater.[35, 114]

If the preheater is omitted from the ante-combustion train, the rubber tubing connecting the various parts of the apparatus must be specially prepared or aged, and a number of directions in regard to such treatment have been given by several investigators. F. Pregl[106a, 113] suggests the heating of the rubber tubing at 100 to 110° C. for several hours while air passes through the tubing. The treatment of rubber tubing with strong potassium hydroxide on a steam bath,[41b] or steaming out until the tubing is odorless, has been suggested as another remedy. M. Boetius[18a] and J. Lindner[77, 78] minimize the rubber tubing used in the ante-combustion train as a source of error.

Pressure Regulator. The original simple pressure regulator described in this manual has been retained by most investigators; the attachment of a drying tube to the outlet of the pressure regulator has been found advantageous.[19b, 97] A pressure regulator based upon the principle of measuring gas velocity [108] has been described by A. Friedrich [41d] and others.[49, 77, 78, 112, 135] The apparatus consists of horizontal thick-walled capillary tubing with two stopcocks provided with edgings for precise adjustment. A capillary U-tube, half filled with colored paraffin oil, is sealed to the horizontal flow tube in such a way that one arm of it is situated between the two stopcocks. The pressure existing in the system is read on a scale which shows the difference in the niveau of the two meniscuses of the paraffin oil. The use of a gas reduction valve for flow control has been advocated by J. Lindner,[77, 78] while W. H. Hamill [49] and J. E. Vance [135] proposed the use of a manometer for the same purpose.

Bubble Counter and U-Tube. The original form of the combined bubble counter and U-tube has been generally retained, although an enlarged type is usually preferred.[97] Modified devices for a similar purpose [19b] as well as complete omission [41i] of this convenient gas flow indicator have been proposed.

Combustion Tube. Changes in the dimension and size of the combustion tube for micro work have been proposed only in one instance.[39] Combustion tubes made of transparent quartz or Supremax glass are preferred. Metal combustion tubes have also been suggested.[5] Attachment of a side arm to the combustion tube has been suggested by E. Müller and H. Willenberg [83] and has by now been universally adopted.[79, 97]

Combustion-Tube Fillings. The so-called *universal* combustion-tube filling suggested and employed by F. Pregl [106, 113] was the subject of a number of investigations, notably by M. Boetius,[18] C. Weygand,[143, 144] and J. Lindner.[77, 78] All possible sources of errors have been investigated and advice about overcoming them has been given.

The asbestos used in combustion-tube fillings was found to be least responsible for disturbances, except that it has the tendency to swell upon heating, thereby affecting the velocity of the gas flow. The swelling may increase the volume of the asbestos by as much as 70%, a fact which has to be taken into consideration when filling the combustion tube.

The copper oxide, which may be activated,[119] has to be examined for basic and volatile constituents; the former can be removed by treatment with acetic acid, followed by strong ignition in open air. The use of lead chromate in combustion-tube fillings has been discontinued.[95, 114]

Commercial silver wool, which has been found to be an adequate absorbent not only for halogen but also for the oxides of sulfur, is usually satisfactory. Possible traces of grease are removed by heating. The silver wool may be replaced with electrolytically prepared finely divided silver powder.[80]

The most serious source of error is the lead peroxide,[9, 28, 41, 46, 66, 142–144] and consequently the directions given by F. Pregl [106a, 113] for its preparation must be followed implicitly. M. Boetius [19c] carried out a number of experiments in regard to the absorption and elimination of water from this reagent and finally recommended the combustion of a substance high in hydrogen content from time to time to re-establish equilibrium. Granular lead peroxide is preferred. A. Friedrich [41d, e] finds that carbon dioxide is also gradually absorbed by the lead peroxide and then given off again when substances containing nitrogen, halogen, or sulfur are combusted. The mechanism of the absorption by lead peroxide of the various oxides of nitrogen formed during the combustion of nitrogen-containing substances has been studied by W. R. Kirner,[65] who showed that the absorption proceeds in accordance with the following equation:

$$2NO + 2PbO_2 \rightarrow Pb(NO_2)_2 \cdot PbO + \tfrac{1}{2}O_2$$

$$2NO_2 + 2PbO_2 \rightarrow Pb(NO_3)_2 \cdot PbO + \tfrac{1}{2}O_2$$

Complete elimination of the lead peroxide from combustion-tube fillings by substituting metallic copper,[33, 98] or silver,[69] or "Hopcalite"[28] has been attempted.

In this manual four combustion-tube fillings have been described, i.e.: a *simple* and a *combination* filling and a *simple band* and a *combination band* filling. Use of the simple combustion-tube filling is indicated in teaching, where the choice of the sample is up to the instructor. This type of combustion-tube filling is applicable to any substance *not* containing nitrogen; it has the advantage that it does not contain lead peroxide, and consequently all disagreeable features of this reagent are eliminated. The simple band filling is necessary when substances of the sterol group, or substances possessing highly condensed ring systems with angular alkyl groups, have to be analyzed. These types of substances invariably gave low results when analyzed with the F. Pregl [106a, 113] so-called *universal combustion* tube filling. The other two fillings are adaptations of the simple and the simple band fillings to organic substances containing nitrogen, when the use of lead peroxide becomes necessary.

The introduction of gold wire into the combustion tube becomes necessary in the combustion of substances containing mercury.[45, 56, 138]

The influence of other elements has been studied by F. C. Silbert and W. R. Kirner [126] and by H. Roth.[112]

Radical changes in the combustion-tube filling have been proposed by P. Haas and F. Rappaport [47] and by G. Ware-Wellwood,[141] who suggested the use of cerium dioxide. P. L. Kirk and A. McCalla [63] recommend manganese dioxide for the same purpose. It is claimed that by the use of this reagent the life of the combustion tube is considerably prolonged. The use of platinum, either as gauze or in the form of platinized asbestos, and the use of palladium asbestos [65] have been found advantageous.

Absorption Tubes. The weighing errors caused by various influences (humidity, changes in atmospheric pressure, use of various types of tares, etc.) upon absorption tubes have been repeatedly investigated.[19, 36, 41f, 51, 56, 77, 78, 104, 111, 121, 128, 137, 143] The original absorption tubes as devised by F. Pregl [106a, 113] and as standardized by H. Lieb and A. Soltys [74] have been found entirely satisfactory, particularly when used with an aspirator.[97] If the precision absorption tubes as recommended by the latter are not available, the introduction of fine metal wires (copper) into the capillary constrictions during the weighing period is of decided advantage,[19e, 97] particularly in humid weather. The use of a control absorption tube was suggested by A. Friedrich,[41f] while J. B. Niederl and E. M. Livingstone [93] recommend the use of a *tare* absorption tube to minimize the influence of unfavorable atmospheric conditions.

The formation of electrostatic charges on the absorption tubes during the wiping process in dry weather is observed very frequently, and several remedies which include the use of ultraviolet light,[111] or a high-frequency discharge,[137] or the drawing of the absorption tube through a flame [128] have been suggested. It was found that a humidity of 60 to 70%,[51] which can easily be maintained in any laboratory, is desirable. Absorption tubes made of Pyrex glass cannot be used.

The use of *Anhydrone* [7] (magnesium perchlorate) for the absorption of water has been widely adopted,[97] although calcium chloride,[106a,112] Dryerite (anhydrous calcium sulfate),[35, 50] as well as phosphorus pentoxide [19d, 32, 134] are still in vogue. Similarly, *Ascarite* [131] (solid sodium hydroxide on asbestos) has replaced [97] the soda-lime [106a, 113] used previously.

The use of a double set of absorption tubes has been found of advantage in series of carbon and hydrogen determinations.[97] Absorption tubes provided with ground-glass joints, thus obviating the use of rubber connections, have been described by K. Bürger [23] and G. L. Royer and co-workers.[114]

For combustion procedures in which the temperature and pressure conditioning *aspirator* [97] is omitted, sealable absorption tubes have repeatedly been proposed. The first of these types were the vertical absorption tubes of F. Blumer,[16] which were subsequently improved by J. V. Dubsky,[33] B. Flaschenträger,[39] K. Lindenfeld,[76] and others.[134] G. Kemmerer and L. T. Hallett [60] suggested horizontal absorption tubes provided with a mercury seal. These types of tubes were subsequently improved by R. T. K. Cornwell [27] and R. J. Robinson and D. J. Doan.[110] Successful replacement of the mercury seal by steel ball valves was reported by I. B. Johns.[59] Several types of mechanically sealable absorption tubes have been described by A. Friedrich.[41c, f] Of these the one designed by E. Abrahamczik [1] proved most successful and has been repeatedly studied.[26, 105] R. O. Clark and G. H. Stillson [26] have enlarged the Abrahamczik types of absorption tubes. These types of absorption tubes have to be opened to the atmosphere for the equalization of pressure, and only when counterpoised with a similar absorption tube do they show greater constancy in weight than the open Pregl absorption tubes [106a, 113] used without an aspirator. "Lefcoseal" stopcock grease is used to lubricate the ground-glass joint.

Rubber Tubing and Connections. The heavy-walled rubber tubing employed for the connections between the absorption tubes has to be specially aged.[41, 106a, 113] The hygroscopicity of this type of rubber tubing has been found to be considerable, and storage in a desiccator of phosphorus pentoxide has therefore been recommended.[18]

Heating Mortar. The original heating mortar as devised by F. Pregl,[106a, 113] underwent a number of changes and improvements.[72, 138] At present the all-glass heating mortar of A. Schöberl [72] seems to incorporate all the advantages of previously designed devices. Scores of electrically heated heating mortars have been devised and are on the market.[61, 62, 117, 122]

Mariotte Flask. The Mariotte flask for measuring the volume of the combustion gases has been improved by the introduction of a stopcock [68, 97, 133] and automatic refilling devices,[6] and has been retained by most investigators.[68, 97, 133]

Heating Units. The long burner has been superseded by equivalent electric heating devices of various designs.[3, 39, 44, 48, 84, 117] Electric furnaces with platinum wiring and movable in all directions [3] have been found very practical. For heating the sample an ordinary gas burner, which allows close observation of the combustion process, is preferred, although replacement of this burner by electric heating units [61, 62, 117, 122] with automatic movements [48, 62, 107, 114] has been proposed.

TREATMENT OF THE SAMPLE

The directions for the preparation and the weighing of the sample, which *must* be a *pure* organic compound, as otherwise the analytical results become meaningless, have been given under the appropriate chapter; they include directions for solids (p. 42), liquids (p. 46), and hygroscopic substances (p. 48). Experiences and directions for carrying out a large number of analyses have been described by M. S. Sherman and R. T. Milner,[125] while C. Tiedcke [132] stated directions and precautions for the analysis of explosive substances. For substances which sublime easily the "technic of the two boats" [36b] is employed to advantage. Novel calculation tables for the direct calculation of the carbon and hydrogen content of organic compounds have been devised by Y. Asahina and S. Bannai.[4] Experience with the Pregl micro carbon and hydrogen apparatus in the tropics has been related by M. C. Nath.[88]

MODIFICATIONS

A *catalytic dry combustion* method following the principle of M. Dennstedt [30] was devised by A. Friedrich.[41c] The combustion is carried out in oxygen, and the lead peroxide is placed in two separate boats. This procedure has the advantage that the lead peroxide may be readily exchanged when exhausted. In this method, a platinum contact, 10 cm. long and 5 cm. wide and folded to form a spiral, serves as a catalyzer in the oxidation. The exclusive use of oxygen, without employment of an aspirator, necessitates sealable absorption tubes.[1, 41c, f] An asbestos plug, introduced at the end of the combustion tube, serves for the establishment of the excess pressure necessary during the combustion. The danger of incomplete combustion nevertheless exists, necessitating suitable changes in the combustion procedure, such as inverted slow combustion of the substance.

Combustion in an *atmosphere of nitrogen* without the use of lead peroxide was studied by J. B. Niederl and J. B. Whitman,[98] who mixed the substance with an excess of finely powdered copper oxide to achieve complete combustion.

A dry combustion method in which the carbon is determined gravimetrically in the form of *barium carbonate* has been described by J. B. Niederl and J. R. Meadow.[94] The combustion gases are passed into a suitably constructed filter absorption tube. The barium carbonate precipitate is filtered in a closed system under exclusion of the atmosphere and is then dried and weighed as usual. This method is too complicated for everyday use, but it permits finer differentiation in the carbon values and reduction of the amount of sample used for analysis to 0.5 mg.

or less. Two-tenths of a milligram of substance could still be analyzed quantitatively by this method to within ±1%.

A *titrimetric dry combustion* method for the determination of *carbon* has been devised by R. B. Schmitt and J. B. Niederl [121] and by R. H. Nagel.[85] A suitably constructed absorption-titration vessel, provided with a spiral gas-inlet tube and an automatic drain arrangement,[85] is attached to the usual combustion tube. Into the same vessel extend the capillary delivery tubes of the 0.1 N barium hydroxide solution, an excess of which is used for the quantitative absorption of the carbon dioxide, and the 0.05 N acid hydrochloric acid, which is used for the back titration of unused alkali. The gas-outlet tube of this titration vessel is attached to a Mariotte flask in the usual manner, and consequently the combustion, absorption, and titration are carried out in a closed system.

A *titrimetric dry combustion* method for the determination of both *carbon and hydrogen* has been described by J. Lindner.[77, 78] The hydrogen is determined as hydrogen chloride, formed by the interaction of the water formed in the combustion with naphthyl phosphorus oxychloride as follows:

$$C_{10}H_7POCl_2 + 2H_2O = C_{10}H_7PO(OH)_2 + 2HCl \tag{1}$$

$$C_{10}H_7POCl_2 + C_{10}H_7PO(OH)_2 = 2C_{10}H_7PO_2 + 2HCl \tag{2}$$

Each molecule of water thus forms two molecules of hydrogen chloride, corresponding to the formation of one mole of hydrogen chloride for each hydrogen atom. The hydrochloric acid formed is determined titrimetrically. The carbon dioxide is absorbed in an excess of 0.1 N barium hydroxide solution, and the unreacted alkali is titrated back with 0.05 N hydrochloric acid solution.

A dry combustion method for the simultaneous determination not only of carbon and hydrogen but also of oxygen has been described by W. R. Kirner [65] and others.[53, 77, 78, 134]

WET COMBUSTION METHODS

H. Lieb and co-workers [73, 118] have devised a micro *wet combustion method for carbon,* which is based upon the earlier macro procedures of M. Nicloux and A. Boivin [20, 89] and the semi-micro procedure of H. Dieterle.[31] The substance is oxidized in a solution of potassium and silver dichromate in concentrated sulfuric acid. The resulting combustion gases are further catalytically combusted in a stream of oxygen and then are absorbed in a suitably constructed titration vessel possessing a glass fritter gas distribution inlet tube. For the absorption of the carbon dioxide an excess of 0.1 N barium hydroxide is used, the unused alkali being titrated with 0.05 N hydrochloric acid solution. Suitable modification in the oxidation vessel for the introduction of capillaries allows also the combustion of liquid substances.[118]

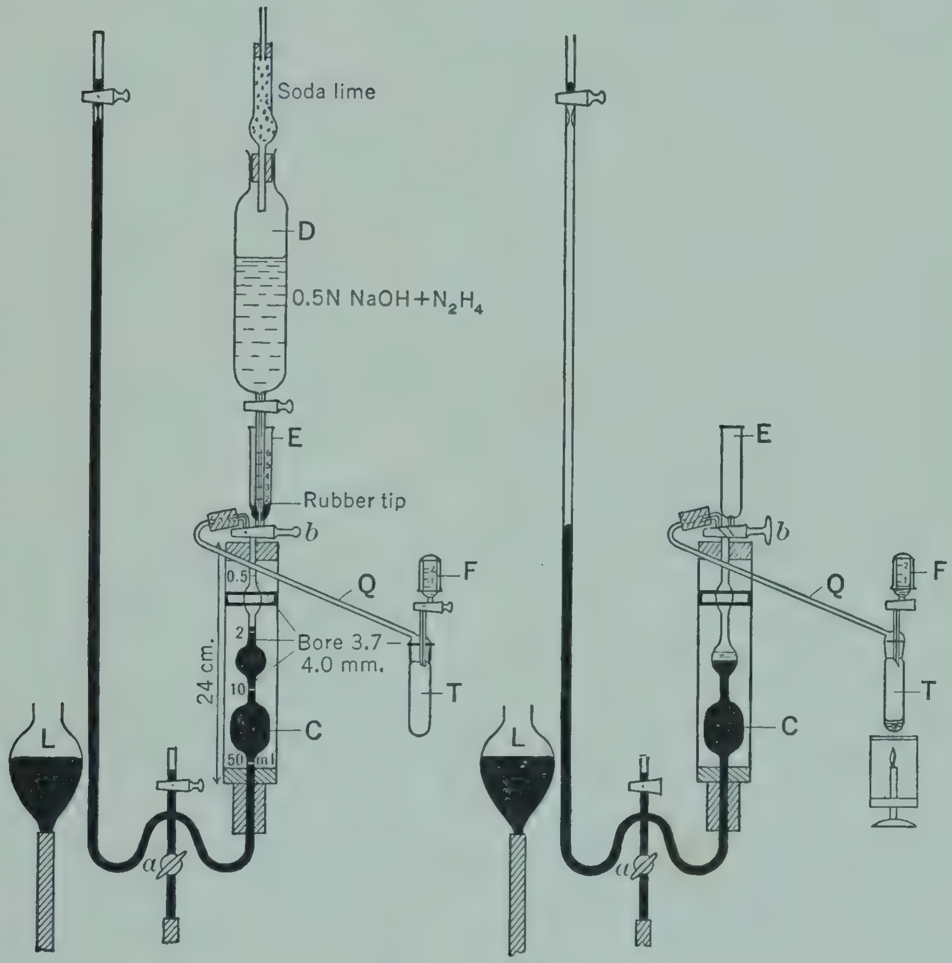
A series of somewhat similarly constituted semi-micro wet combustion methods have been devised. In most of them potassium or silver dichromate, or both,[2, 20, 40, 64, 89, 103] is used as the oxidizing agent. Since this agent alone does not always ensure complete combustion, the combustion gases are usually subjected to a catalytic after-combustion.[25, 102] Instead of dichromates, potassium permanganate,[86] potassium iodate,[25, 127] and persulfate [109] have been suggested as oxidizing agents.

Gas Volumetric and Manometric Methods

Several types of gas-volumetric methods for the determination of carbon alone [8, 13, 29, 136] or of carbon and hydrogen and also of simultaneous determination of carbon, hydrogen, and nitrogen,[11, 15] carbon and oxygen,[24] and carbon, nitrogen, and oxygen [67] have been devised. The procedures may involve dry as well as wet combustion methods, with either volumetric [8, 29] or manometric [136] determination of the combustion gases.

In the *manometric wet combustion method* for carbon as devised by D. D. Van Slyke and J. Folch [136a] the combustion mixture, which effects quantitative oxidation of the sample in one to three minutes, consists of fuming sulfuric, phosphoric, chromic, and iodic acids. The carbon dioxide formed is collected and measured in the Van Slyke-Neill manometric apparatus (Fig. 35, I and II), which consists essentially of a digestion tube, the carbon dioxide absorption chamber, and the manometer. The combustion mixture is prepared by adding 167 ml. of syrupy phosphoric acid (sp. gr.: 1.7) and 333 ml. of fuming sulfuric acid (20% sulfur trioxide) to 25 g. of chromium trioxide (chromic acid anhydride) and 5 g. of potassium iodate contained in a 1-liter Pyrex Erlenmeyer flask provided with a ground-glass stopper. The absorption liquid consists of carbonate-free 0.815 N sodium hydroxide solution to which hydrazine sulfate (2 g. for each 100 ml. solution) has been added.

The actual combustion is carried out as follows: Into the digestion tube containing the weighed sample are introduced several pieces of Alundum and 200 mg. of potassium iodate. With a medicine dropper a thick ring of syrupy phosphoric acid is then drawn around the upper part of the ground-glass joint of T (Fig. 35, I), while T is in a horizontal position. Cup F of connecting tube Q is filled to the 2-ml. mark with fresh combustion fluid, and the stopper is then fitted to the digestion tube containing the sample. The digestion tube is now connected with chamber *C*, as shown in Figs. 35, I and II, chamber *C* being completely filled with mercury. Cock *b* (Fig. 35, I) is turned to connect *C* and *T*, and the mercury in *C* is lowered to the 50-ml. mark. This procedure draws about two-thirds of the air from *Q* and *T* over into *C*. Cock *b* is then closed, mercury is readmitted into *C*, the air trapped over the mercury in *C* is ejected through cup *E*, and cock *b* is then closed again.



I. II.

FIG. 35.

After this, 2 ml. of the alkali-hydrazine solution is measured from *D* into *C* through a mercury seal, as shown in Fig. 35, I. The admission of the alkali is best regulated by opening cock *b* wide and controlling the flow of mercury through cock *a*. The admission is stopped when the mercury in *C* falls to a level of about 1 mm. above the 2-ml. mark, as shown in Fig. 35, I. Then *D* is withdrawn and mercury from *E* is let into the chamber to fill the capillaries of cock *b*. The displacement of the slight volume of solution from the capillary of *b* into the chamber brings the volume of solution in *C* to exactly the 2-ml. mark. Cup *C* is rinsed with acidified water; this cup is never permitted to stand with its walls wet with alkali.

Then *L* is lowered, and mercury is drawn out of *C* and the manometer until the mercury in the manometer is about level with the 2-ml. mark on *C*. Cock *a* is then closed, and cock *b* is turned to connect chamber *C* with the combustion tube. The leveling bulb is then placed, as shown in Figs. 35, I and II, at such a height that the mercury surface in it is about level with the 50-ml. mark on *C* and remains at this level until the combustion is finished. A measured volume of combustion fluid is now run from *F* into *T*; for combustions with 2 to 3.5 mg. of carbon 2 ml. of the fluid is used. The microflame is now brought under the combustion tube, and the arrangement is as shown in Fig. 35, II.

Fine bubbles of carbon dioxide gas begin to rise in the combustion fluid as soon as the fluid is warmed. A minute or so is taken to warm it to boiling. Preferably the evolution of carbon dioxide should not be so rapid that at any time it makes a foam collar more than 2 cm. high on the fluid, although even if it should fill the greater part of the tube, the analysis is not necessarily lost. After the initial carbon dioxide evolution the fluid is heated rapidly to boiling.

As carbon dioxide and oxygen are evolved, the mercury falls in *C* (Fig. 35, I) and rises in the manometer. Cock *a* is slightly opened every few seconds at this stage to admit mercury from *L* into *C* and keep the gas space in *C* at about 1 ml. Within about a minute from the beginning of heating enough gas has been evolved to press the mercury in the manometer up to its top, and to permit complete opening of cock *a* without causing backflow of alkali solution from *C* to *T*. Cock *a* is now left fully open during the rest of the combustion, and the boiling proceeds quietly at about 150 mm. less than atmospheric pressure. Vigorous boiling at about 600 mm. pressure is continued, with foam filling one-third to one-half of the tube, for 1.5 minutes to complete the combustion.

After the combustion is completed, the flame is left under the tube exactly as during the combustion, while the mercury in *C* (Fig. 35, I) is lowered and raised 20 times to cause complete transfer of carbon dioxide to the alkali solution in *C*. The 20 excursions should take about three minutes. At each lowering of the leveling bulb the mercury in *C* is dropped to about the 50-ml. mark, and the fluid in *T* boils vigorously. The tube should then fill with foam. At each raising the bulb is lifted till the gas space in *C* is compressed to about 5 ml. After 5, 10, 15, and 20 excursions of the mercury in this manner, the respective percentages of the carbon dioxide transferred to the alkali solution in *C* have been found to be 91, 98.3, 99.7, and 100.

After the absorption is completed, the flame is removed from the combustion tube, cock *b* (Fig. 35, I) is closed, and tube *Q* is disconnected from the chamber. The hot combustion tube is removed with tongs or a strong metal test-tube holder.

Before oxygen and nitrogen are ejected, the curved inlet capillary above cock *b* is filled with mercury drawn in from a small bottle. About as much mercury is left in this capillary above the cock as is shown in Fig. 35, I. Then, with cock *b* closed, the gases in *C* are put under positive pressure by raising leveling bulb *L* a little above cock *b*. With the bulb at this level, cock *a* is closed and *b* opened to connect chamber *C* with cup *E* above it. Mercury is then admitted from *L* into chamber *C* until the rising alkali solution, driving the gases out through *E*, just reaches the bottom of cock *b*. In succession cocks *a* and *b* are then closed. The leveling bulb is lowered to the position shown in Figs. 35, I and II, and a little mercury is admitted from cup *E* into the chamber to seal the connecting capillary. A small bubble of air, trapped in the capillary, is thus readmitted into *C*, but it has no influence on the carbon dioxide determination, since it is free of carbon dioxide.

Exactly 1 ml. of 2 *N* lactic acid is measured into chamber *C* from an accurate stopcock pipet provided with a rubber-ringed tip. The admission is made through a mercury seal in the same manner shown in Fig. 35, I, for the admission of alkali. Mercury to fill the connecting capillary between *E* and *C* is admitted after the acid.

The mercury in the chamber is then lowered to the 50-ml. mark, cock *a* leading to the leveling bulb is closed, and the chamber is shaken twenty or thirty seconds, most of the carbon dioxide being extracted from the solution. The carbon dioxide that has entered the gas phase increases the pressure there enough to force the mercury down below the 50-ml. mark. To correct for this displacement, enough mercury is admitted from the leveling bulb to bring the top of the mercury meniscus in the chamber exactly to the 50-ml. mark. The chamber is then shaken for 1.5 minutes to complete the extraction of the carbon dioxide from the solution.

Mercury is now admitted from the leveling bulb until the volume of the gas phase in the chamber is reduced to 10 ml. As the meniscus of the solution in the chamber approaches this mark it is desirable to watch it with a magnifying lens in order to stop it exactly on the line.

The reading p_1 is then taken on the manometer. After this the cock leading to the leveling bulb is opened, while the bulb is left at the level as shown in Figs. 35, I and II, so that the gas in the chamber is under slight negative pressure. Into cup *E* is measured 0.5 ml. of 5 *N* sodium hydroxide solution, and the alkali is then admitted into the chamber. When all the alkali is in the chamber, 2 or 3 ml. of acidified water is poured into the cup, followed by about 0.5 ml. of mercury. The mercury is then run into the chamber, dislodging any of the alkali solution which may have adhered in the part of the chamber under cock *b*.

To mix the solutions in the chamber, and to insure absorption of the last traces of carbon dioxide, the mercury in the chamber is raised and lowered three times, each lowering bringing the surface of the solution in the chamber, not to its bottom, but only to a point a little below the 10-ml. mark, and each raising bringing the pressure up to about atmospheric.

The solution meniscus in the chamber is then brought to a point a little below the 10-ml. mark at which p_1 was read, and is allowed to stand there for one minute while the solution drains down from the walls above the mark. During this time the temperature in the water jacket of the chamber is read and the corresponding carbon factor ascertained. Then the meniscus is raised exactly to the 10-ml. mark and reading p_2 is made on the manometer. The pressure, P_{CO_2}, of carbon dioxide from the combustion is calculated as $P_{CO_2} = p_1 - p_2 - c$, where c is the value of $p_1 - p_2$ as obtained in a blank analysis.

The milligram of carbon in the sample are calculated by the factors as given in the table below, which may be corrected if necessary by a factor obtained from control analyses of a standard pure substance.

$$\text{Milligrams carbon} = P_{CO_2} \times \text{factor}$$

FACTORS FOR CARBON CALCULATION

Temperature ° C.	*Factors* ($a = 10.00$; $s = 3.00$; $i = 1.007$)
20	0.006954
21	922
22	890
23	859
24	828
25	798
26	769
27	740
28	711
29	683
30	655

To clean the chamber for the next analysis the used alkaline lactate solution is ejected and the chamber is washed once with dilute acid and once with water. A rapid and convenient technic is the following: The mercury leveling bulb is lowered about 80 cm. below chamber C (Fig. 55, I) so that all mercury will drain out of the chamber. While it is draining, a few drops of the 2 N lactic acid are placed in the cup at the top of the chamber, together with enough water to fill the cup. The acidified water (but no air) is now let into the evacuated chamber. The leveling bulb is then raised and the solution expelled from the chamber. The washing is then repeated in the same manner, except that only distilled water is used. After the water is ejected, the mercury is lowered once more to the bottom of the chamber, and is allowed to rise rather slowly, so that the film of water adherent on the sides of the chamber is detached and floats up on the mercury. The drop of water thus collected, together with about 1 ml. of mercury, is run up into the cup, and the apparatus is ready for the next combustion.

SEMI-MICRO DRY COMBUSTION METHODS

Semi-micro dry combustion methods are usually compromises between the classical Liebig macro combustion method and the Pregl micro combustion method, approximately 200 mg. of sample being used in the former and about 4 mg. in the latter. In order to incorporate the obvious advantages of the micro method in regard to sample size and speed of analysis into combustion procedures, meso- and semi-macro, meso- and semi-micro combustion procedures have been devised.[11–14, 17, 21, 25, 30, 33, 43, 46, 54, 70, 71, 87, 99–101, 116, 129, 130, 140, 148]

The necessity of using a microanalytical balance has been felt to be a distinct drawback in micro combustion work. Consequently J. B. Niederl and co-workers[96] investigated possible replacement of the microanalytical balance by an ordinary analytical balance in such combustion analyses. It was found that, with the apparatus described in this manual, samples of 1 to 15 mg. in weight can be analyzed *without* any changes in the apparatus or procedure, except that the sweeping-out process of the combustion gases has to be proportioned to the amount of sample used (100 ml. for milligram samples and 150 ml. for decamilligram samples). Merely the size of the sample needs to be enlarged in proportion with the precision of the ordinary analytical balance used; this corresponds approximately to 1 mg. of sample for each precision microgram of the balance. Incidentally, the early combustion analyses of F. Pregl[106c] in 1911 and subsequently by L. E. Wise[146] in 1917 had been carried out in much the same manner.

LITERATURE

1. Abrahamczik, E., *Mikrochemie*, **22**, 227 (1937).
2. Adams, J. E., *Ind. Eng. Chem., Anal. Ed.*, **6**, 277 (1934).
3. American Platinum Works, Newark, N. J., U. S. A.
4. Asahina, Y., and Bannai, S., "Rechen-Tabelle, von C- und H-Gehalt der organischen Verbindungen," Tokyo, Japan, 1936.
5. Avery, S., Brackenbury, J., and Maclay, W., *Ind. Eng. Chem., Anal. Ed.*, **4**, 238 (1932).
6. Backeberg, O. G., *Mikrochemie*, **21**, 135 (1936).
7. Baker, J. T., Chemical Co., Phillipsburg, N. J., U. S. A.
8. Ball, T. Z., Huffman, E. W. D., and Degering, E. F., Rochester Meeting, Am. Chem. Soc., September, 1937.
9. Baxter, G. P., and Hale, A. H., *J. Am. Chem. Soc.*, **58**, 510 (1936).
10. Baxter, G. P., and Starkweather, H., *J. Am. Chem. Soc.*, **38**, 2036 (1916).
11. Berger, H., *J. prakt. Chem.*, **133**, 1 (1932).
12. Berl, E., and Burkhardt, H., *Ber.*, **59**, 890 (1926).
13. Berl, E., Schmidt, A., and Koerber, W., *Ind. Eng. Chem., Anal. Ed.*, **12**, 245 (1940).
14. Berl, E., Schmidt, A., and Winnacker, K., *Ber.*, **61**, 83 (1928).
15. Berraz, G., *Anales inst. investigaciones cient. tecnol.* (*Argentina*), **7**, 70 (1937).
16. Blumer, F., *Ber.*, **50**, 1710 (1917).
17. Bobranski, B., and Sucharda, E., *Mikrochemie*, **7**, 278 (1929).
18. Böck, F., and Beaucourt, K., *Mikrochemie*, **6**, 133 (1928).
19. Boetius, M., "Über die Fehlerquellen in der mikroanalytischen Bestimmung des Kohlen- und Wasserstoffes nach der Methode von Fritz Pregl," Verlag Chemie, Berlin, 1931; (*a*) p. 27; (*b*) p. 36; (*c*) p. 59; (*d*) p. 82; (*e*) p. 100.
20. Boivin, A., *Compt. rend.*, **187**, 1076 (1928).
21. Brodie, S. S., *Ind. Eng. Chem., Anal. Ed.*, **11**, 517 (1939).
22. Bruce, W. F., *Mikrochemie*, **18**, 103 (1935).
23. Bürger, K., *Ber.*, **72**, 40 (1939).
24. Christensen, B. E., and co-workers, *J. Am. Chem. Soc.*, **61**, 3001 (1939); *Ind. Eng. Chem., Anal. Ed.*, **8**, 194 (1936); **9**, 59, 293, 400 (1937); **12**, 364 (1940); **13**, 444 (1941).
25. Clark, E. P., *J. Assoc. Official Agr. Chem.*, **16**, 255 (1933).
26. Clark, R. O., and Stillson, G. H., *Ind. Eng. Chem., Anal. Ed.*, **12**, 494 (1940).
27. Cornwell, R. T. K., *Ind. Eng. Chem., Anal. Ed.*, **3**, 4 (1931).
28. Corwin, A. H., Rochester Meeting, Am. Chem. Soc., September, 1937; *Mikrochemie*, **24**, 98 (1938).
29. Degering, E. F., and Ball, T. Z., *Ind. Eng. Chem., Anal. Ed.*, **12**, 124 (1940).
30. Dennstedt, M., *Ber.*, **30**, 1590 (187); *Z. anal. Chem.*, **42**, 417 (1903). "Anleitung zur vereinfachten Elementaranalyse," V. Meissner's Verlag, Hamburg, 1919.
31. Dieterle, H., *Arch. Pharm.*, **262**, 35 (1924).
32. Drew-Keith, H. D., and Porter, C. R., *Mikrochemie*, **7**, 149 (1929).
33. Dubsky, J. V., "Vereinfachte quantitative Mikroelementaranalyse organischer Substanzen," Veit and Co., Leipzig, 1917; *Z. anal. Chem.*, **59**, 254 (1920).
34. Eastman Kodak Co., Rochester, N. Y., U. S. A.
35. Elek, A., Rockefeller Institute for Medical Research, New York, N. Y. (*a*) private communication; (*b*) *Ind. Eng. Chem., Anal. Ed.*, **10**, 51 (1938).

36. Evans, R. N., Davenport, J. E., and Revukas, A. J., *Ind. Eng. Chem., Anal. Ed.*, **11**, 553 (1939).
37. Fieser, L. F., and Jacobsen, R. P., *J. Am. Chem. Soc.*, **58**, 943 (1936).
38. Fisher, H. L., "Laboratory Manual of Organic Chemistry," 4th Edition, John Wiley & Sons, New York, N. Y., 1938, p. 328.
39. Flaschenträger, B., *Z. angew. Chem.*, **36**, 481 (1923); **41**, 840 (1928); **39**, 717 (1926); *Mikrochemie*, **9**, 15 (1931); *J. prakt. Chem.*, (2) **99**, 34 (1919).
40. Friedmann, T. E., and Kendall, A. I., *J. Biol. Chem.*, **82**, 45 (1929).
41. Friedrich, A., (*a*) "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, p. 22. (*b*) *Mikrochemie*, **9**, 20 (1930); (*c*) *ibid.*, **10**, 329 (1931); (*d*) *ibid.*, **10**, 338 (1931); (*e*) *ibid.*, **10**, 342 (1931); (*f*) *ibid.*, **19**, 23 (1935); (*g*) *ibid.*, **23**, 129 (1937). (*h*) *Z. anal. Chem.*, **74**, 412 (1928). (*i*) *Z. angew. Chem.*, **36**, 481 (1923).
42. Friedrich, A., and Sternberg, H., *Mikrochemie, Molisch Festschrift*, 1936, p. 118.
43. Funk, C., "Mikroanalyse nach der Mikro-Dennstedt-Methode," Munich, 1925.
44. Fünner, W., *Mikrochemie*, **10**, 66 (1931).
45. Furter, M., *Mikrochemie*, **9**, 27 (1931).
46. Gränacher, Ch., *Helv. Chim. Acta*, **10**, 449 (1927); *Z. anal. Chem.*, **74**, 409 (1928).
47. Haas, P., and Rappaport, F., *Mikrochemie*, **7**, 327 (1929).
48. Hallett, L. T., Rochester Meeting, Am. Chem. Soc., September, 1937; *Ind. Eng. Chem., Anal. Ed.*, **10**, 101 (1938); "Quantitative Microchemical Analysis," Scott's Standard Methods of Chemical Analysis, D. Van Nostrand Co., New York, N. Y., 1939, pp. 2460–2547.
49. Hamill, W. H., *Ind. Eng. Chem., Anal. Ed.*, **9**, 355 (1937).
50. Hammond, W. A., Drierite Co., Yellow Springs, Ohio; *Ind. Eng. Chem., News Ed.*, **18**, No. 23, 1103 (1940).
51. Hayman, D. F., *Ind. Eng. Chem., Anal. Ed.*, **8**, 342 (1936). Rochester Meeting, Am. Chem. Soc., September, 1937.
52. Hecht, F., *Mikrochim. Acta*, **1**, 194 (1937).
53. Hennig, H., *Chem. Fabrik*, **9**, 239 (1936).
54. Hepner, B., and Pojas, M., *Compt. rend.*, **1937**, 397.
55. Heraeus, W. C., Hanau, a. M., Germany.
56. Hernler, F., *Mikrochemie, Pregl Festschrift*, 1929, p. 154.
57. Hohl, H. V., *Mikrochemie*, **13**, 189 (1933).
58. Huffman, E. W. D., *Ind. Eng. Chem., Anal. Ed.*, **12**, 53 (1940).
59. Johns, I. B., *Mikrochemie*, **24**, 217 (1938); **25**, 382 (1938); Dallas Meeting, Am. Chem. Soc., April, 1938.
60. Kemmerer, G., and Hallett, L. T., *Ind. Eng. Chem.*, **19**, 173 (1927).
61. Kirby, H., *Chemistry & Industry*, 1939, p. 117.
62. Kirchenbauer, J., Singen bei Pforzheim, Germany.
63. Kirk, P. L., and McCalla, A., *Mikrochemie*, **12**, 88 (1932).
64. Kirk, P. L., and Williams, P. A., *Ind. Eng. Chem., Anal. Ed.*, **4**, 403 (1932).
65. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **5**, 363 (1933); **7**, 366 (1935); **8**, 57 (1936); **9**, 535 (1937); **10**, 342 (1938); *Mikrochemie*, **24**, 98, 219 (1938).
66. Kopfer, F., *Z. anal. Chem.*, **17**, 28 (1878).
67. Krogh, A., *Biochem. Z.*, **221**, 247 (1930).
68. Kuck, J., College of the City of New York, New York, N. Y., private communication.

69. Kuespert, K. H., *Chem. Fabrik*, **6**, 63 (1933).
70. Labriola, R. A., *Chemia*, **10**, 330 (1937).
71. Lauer, W. M., and Dobrovolny, F. J., *Mikrochemie, Pregl Festschrift*, 1929, p. 243.
72. Lieb, H., *Mikrochemie*, **14**, 263 (1937).
73. Lieb, H., and Krainick, H. G., *Mikrochemie*, **9**, 367 (1931); **10**, 99 (1932).
74. Lieb, H., and Soltys, A., *Mikrochemie*, **20**, 59 (1936); *ibid., Molisch Festschrift*, 1936, p. 290.
75. Lieb, H., and Benedetti-Pichler, A. A., "Mikrochemische Analyse," Berl-Lunge, "Chemisch-technische Untersuchungsmethoden," Vol. I, Eighth Edition, J. Springer, Berlin, 1929, p. 1166.
76. Lindenfeld, K., *Mikrochemie*, **9**, 244 (1931); **16**, 153 (1935).
77. Lindner, J., "Mikromassanalytische Bestimmung des Kohlenstoffes und Wasserstoffes mit grundlegender Behandlung der Fehlerquellen in der Elementaranalyse," Berlin, 1935; *Ber.*, **59**, 2561, 2806 (1926); **60**, 124 (1927); **63**, 949, 1123, 1396, 1672 (1930); **65**, 1696 (1932); **70**, 1025 (1937).
78. Lindner, J., and co-workers, *Z. anal. Chem.*, **66**, 305 (1925); **72**, 135 (1927); **78**, 188 (1929); *Z. angew. Chem.*, **40**, 462 (1927); *Mikrochemie*, **10**, 321, 440 (1931); **20**, 209 (1936); **25**, 197 (1938); *ibid., Emich Festschrift*, 1930, p. 191.
79. Lunde, G., *Biochem. Z.*, **176**, 157 (1926).
80. MacNevin, W., and Clark, H. S., *Ind. Eng. Chem., Anal. Ed.*, **10**, 338 (1938).
81. Meixner, A., and Kröcker, F., *Mikrochemie*, **5**, 121 (1927).
82. Merck, E., Darmstadt, Germany.
83. Müller, E., and Willenberg, H., *J. prakt. Chem.*, (2) **99**, 34 (1919); *Z. anal. Chem.*, **61**, 3 (1922).
84. Müller, R. H., *Ind. Eng. Chem., Anal. Ed.*, **12**, 620 (1940).
85. Nagel, R. H., *Mikrochemie*, **26**, 22 (1939).
86. Nardo, de, *Giorn. chim. ind. applicata*, **10**, 253 (1928).
87. Natelson, S., Brodie, S. S., and Conner, E. B., *Ind. Eng. Chem., Anal. Ed.*, **10**, 609 (1938).
88. Nath, M. C., *Mikrochemie*, **26**, 165 (1939).
89. Nicloux, M., and Boivin, A., *Compt. rend.*, **184**, 890 (1927).
90. Niederl, J. B., *Ind. Eng. Chem., Anal. Ed.*, **7**, 215 (1935).
91. Niederl, J. B., *J. Chem. Education*, **13**, 255 (1936).
92. Niederl, J. B., *Z. anal. Chem.*, **89**, 57 (1932).
93. Niederl, J. B., and Livingstone, E. M., own investigation.
94. Niederl, J. B., and Meadow, J. R., *Mikrochemie*, **9**, 350 (1931).
95. Niederl, J. B., and Niederl, V., *Mikrochemie-Microchim. Acta*, **26**, 28 (1939).
96. Niederl, J. B., Niederl, V., Nagel, R. H., and Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).
97. Niederl, J. B., and Roth, R. T., *Ind. Eng. Chem., Anal. Ed.*, **6**, 272 (1934).
98. Niederl, J. B., and Whitman, J. B., *Mikrochemie*, **11**, 274 (1932).
99. Niemann, C., and Danford, V., *Ind. Eng. Chem., Anal. Ed.*, **12**, 563 (1940).
100. Orthner, L., and Reichel, L., "Organisch-Chemisches Praktikum," Verlag Chemie, Berlin, 1929.
101. Pentschev, N. P., *Z. anal. Chem.*, **113**, 431 (1938).
102. Pettenkoffer, P., Lange, L., and Ambler, H. R., "Technical Gas Analysis," Gwiney and Jackson, London, 1934, p. 221.
103. Pollard, C. B., and Forsee, W. T., *Ind. Eng. Chem., Anal. Ed.*, **7**, 77 (1935).

104. Power, F. W., *Mikrochemie*, **22**, 263 (1937); *Ind. Eng. Chem., Anal. Ed.*, **11**, 660 (1939); Chapel Hill Meeting, Am. Chem. Soc., April, 1937; Milwaukee Meeting, Am. Chem. Soc., September, 1938.
105. Prater, A. N., *Ind. Eng. Chem., Anal. Ed.*, **12**, 184 (1940).
106. Pregl, F., (*a*) "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, pp. 21–90; (*b*) *op. cit.*, p. 19. (*c*) "Abderhalden's Handbuch der biologischen Arbeitsmethoden," Urban L. Schwarzenberg, Vienna and Berlin, 1912, Vol. V, p. 1311–1312.
107. Reihlein, H., *Mikrochemie*, **23**, 285 (1938).
108. Riesenfeld, E. H., *Chem. Ztg.*, **42**, 10 (1918).
109. Rischbieth, P., *Z. physik. chem. Unterricht*, **43**, 132 (1930).
110. Robinson, R. J., and Doan, D. J., *Ind. Eng. Chem., Anal. Ed.*, **11**, 406 (1939).
111. Rodden, C. J., *Ind. Eng. Chem., Anal. Ed.*, **12**, 693 (1940).
112. Roth, H., *Z. angew. Chem.*, **50**, 593 (1937); *Mikrochemie, Molisch Festschrift*, 1936, p. 373.
113. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son and Co., Philadelphia, Pa., 1937, pp. 15–68.
114. Royer, G. L., Norton, A. R., and Sundberg, O. E., *Ind. Eng. Chem., Anal. Ed.*, **12**, 688 (1940).
115. Sakamoto, S., *J. Pharmac. Soc.* (*Japan*), **59**, 623 (1939).
116. Sanchez, J. A., *J. pharm. chim.*, **24**, 297 (1936).
117. Sargent, E. H., and Co., 155–165 E. Superior Street, Chicago, Ill., U. S. A.; *Ind. Eng. Chem., News Ed.*, **18**, No. 23, p. 1104 (1940); *Sargent Magazine*, **1**, 17 (1941).
118. Schadendorf, E., and Zacherl, M. K., *Mikrochemie*, **10**, 99 (1932).
119. Schenk, R., and Kurzen, G., *Z. anorg. allgem. Chem.*, **235**, 97 (1937).
120. Schering-Kahlbaum, A.-G., Berlin, Germany.
121. Schmitt, R. B., and Niederl, J. B., *Mikrochemie*, **24**, 59 (1938); Rochester Meeting, Am. Chem. Soc., September, 1937.
122. Schneider, F. and Van Mater, H. L., *Ind. Eng. Chem., Anal. Ed.*, **9**, 295 (1937).
123. Schoorl, N., *Chem. Weekblad*, **16**, 481 (1919).
124. Sepalowa-Michailowa, L. A., "Microanalytical Determination of Carbon and Hydrogen in Organic Compounds" (Russ.), Goschimtechisdat, 1934.
125. Sherman, M. S., and Milner, R. T., Rochester Meeting, Am. Chem. Soc., September, 1937.
126. Silbert, F. C., and Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **8**, 353 (1936).
127. Stanek, V., and Nemes, T., *Z. anal. Chem.*, **95**, 244 (1933).
128. Sternberg, H., *Mikrochemie*, **22**, 187 (1937); **24**, 65 (1938).
129. Sucharda, E., and Bobranski, B., "Halbmikromethoden zur automatischen Verbrennung organischer Substanzen," F. Vieweg und Sohn, Braunschweig, 1929; *Z. anal. Chem.*, **77**, 462 (1929).
130. Ter Meulen, H., and Heslinga, J., "Neue Methoden der organisch-chemischen Analyse," Akad. Verlagsgesellschaft, Leipzig, 1927.
131. Thomas, A. H., and Co., Philadelphia, Pa., U. S. A.
132. Tiedcke, C., *Mikrochemie*, **16**, 171 (1935); **23**, 241 (1937); **25**, 67 (1938); **28**, 64 (1939); Rochester Meeting, Am. Chem. Soc., September, 1937.
133. Unterzaucher, J. *Mikrochemie*, **18**, 312 (1935).
134. Unterzaucher, J., and Bürger, K., *Ber.*, **71**, 429 (1938).
135. Vance, J. E., *Ind. Eng. Chem., Anal. Ed.*, **13**, 132 (1941).

136. Van Slyke, D. D., and co-workers, *J. Biol. Chem.*, **61**, 523 (1924); **73**, 127 (1927); **102**, 635 (1933); **130**, 545 (1939); (*a*) **136**, 509 (1940).
137. Van Straten, F. W., and Ehret, W. F., *Ind. Eng. Chem., Anal. Ed.*, **9**, 443 (1937).
138. Verdino, A., *Mikrochemie*, **6**, 5 (1928); **9**, 123 (1931).
139. Vetter, F., *Mikrochemie*, **10**, 109 (1932).
140. Wanscheidt, A., and Schupinskaja, M., Arb. VI, *Mendelejev Congr. Theor. Applied Chem.*, 1932, II, p. 316.
141. Ware-Wellwood, G., *Mikrochemie*, **15**, 237 (1934).
142. Weil, H., *Ber.*, **43**, 149 (1910).
143. Weygand, C., "Quantitative analytische Mikromethoden der organischen Chemie in vergleichender Darstellung," Akademische Verlagsgesellschaft, Leipzig, 1931, pp. 70–163.
144. Weygand, C., and Hennig, H., *Chem. Fabrik*, **9**, 8 (1936).
145. White, E. V., and Wright, G. F., *Can. J. Research*, **14**, Sect. B, 427 (1936).
146. Wise, L. E., *J. Am. Chem. Soc.*, **39**, 2055 (1917).
147. Wrede, F., *Ber.*, **55**, 557 (1922).
148. Zappi, E. V., and Labriola, R., *Anales asoc. quím. argentina*, **24**, 47 (1937).

VI. DETERMINATION OF HALOGEN

GRAVIMETRIC METHODS

WET COMBUSTION (CARIUS) METHOD

Principle

The organic substance containing halogen is oxidized in a sealed tube [24, 39, 50, 71, 74, 80a, 87] in the presence of nitric acid at a temperature of 280–300° C., whereby the halogen is converted quantitatively into ionizable halogen which is precipitated and determined gravimetrically as silver halide.

(*a*) $2RX \xrightarrow[\Delta]{HNO_3} X_2 + xCO_2 + H_2O$ (oxidation)

(*b*) $X_2 \xrightarrow{+2AgNO_3} 2AgX$ (precipitation)

Apparatus

Furnace (Fig. 36). The pressure tubes can be heated in any suitably constructed furnace; the one usually employed is a hollow round retort of heavy metal plate supported by a metal stand about 35 cm. high and 28 cm. long and covered at the top and sides with heavy asbestos or Transite plates. The retort has three or more apertures of approximately 1.5-cm. diameter and is heated by a suitable long burner. Electrically heated furnaces may even be provided with clock mechanisms between the relay and the furnace which automatically control the time of heating.

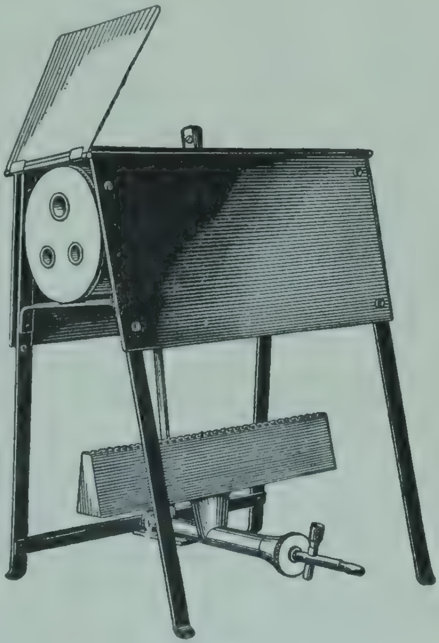


Fig. 36. Furnace.

Pressure Tubes. The pressure tubes are of straight-walled Pyrex glass without a constricted end. They are 20 cm. long, 9 to 10 mm. in inside diameter, and should have a minimum wall thickness of 1 mm. and a round bottom. Tubes which possess a visible seam are unsatisfactory.

Filter Tubes (Fig. 37).[80a, 87] Two types of filter tubes are available for the filtration of the silver halide. A filter of either type is 15 cm. long and possesses a bulb 4 cm. long and 12 mm. wide and a stem 10 cm. long and of 4-mm. outer and 2.5-mm. inner diameter. The first type (*a*) contains an asbestos filter mat in the bulb-shaped portion over a constriction of 0.3-mm. diameter. The newer type (*b*) is supplied with a plate of sintered glass of medium density. To prepare a filter mat of the proper density the asbestos is deposited as follows: The filter tube is placed on the filtration apparatus and a 2-mm. layer of medium-fine Gooch crucible asbestos is filtered on top of the constriction and evenly pressed together with a sharp-edged glass rod; this is followed by a thin layer of fine asbestos and the whole is again pressed together to a mat of 2-mm. total thickness. The asbestos mat is washed first with about 250 ml. of distilled water, several times with hot cleaning solution, and again with distilled water and alcohol. Its orifice is then closed with a dust filter and the tube is dried with slight suction in the drying block at about 120° C. The filter tube is counterpoised with a ground-glass stoppered tare bottle and lead shot.

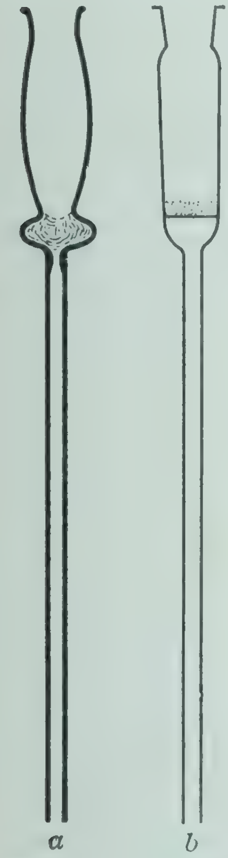


Fig. 37. *a*, Filter Tube with Asbestos Mat; *b*, Filter Tube with Sintered-Glass Plate.

The tube with the sintered glass filter plate (*b*) is preferable because it is easier to clean and the weight seems to show greater constancy. A filter plate of No. 3 porosity will retain the silver halide. However, these filters do not have a uniform porosity, and filters of the same density (No. 3) usually show a rather wide variation in filter speed. Each filter tube should therefore be tested for its speed of filtration as follows: when the filter tube is placed on the filtration apparatus and attached to a good water pump, operating at maximum pressure, it should filter water at a rate of 70 ml. per minute.

The retentivity of a filter tube is checked by filtering a known amount of silver bromide, prepared by precipitating a weighed sample of potassium bromide (approximately 5 mg.), dissolved in 5 ml. of halogen-free distilled water, with 2 ml. of a 5% silver nitrate solution in the presence of 1 ml. of concentrated nitric acid. The precipitated silver bromide is coagulated by heating on a steam bath for several minutes and is then filtered. The filter tube is rinsed, dried, and weighed as usual. Then 50 ml. of water is siphoned through the filter tube, after which the filter is again dried and weighed as before. A satisfactory filter tube should retain the precipitate to within 3 to 5 parts per 1000.

A new fritted glass filter, as well as a used one in which the pores have become clogged, is cleaned by boiling it for 15 minutes in dilute nitric acid (1 : 1), then for the same time in distilled water, and rinsing it repeatedly, five or six times, with distilled water, and with alcohol. After the filter tube has been dried and weighed the procedure of rinsing it several times with distilled water and alcohol is repeated, and the tube is dried and weighed again; the two weighings should agree with each other within ±10 micrograms. The above treatment with dilute nitric acid is important for the achievement of constancy of weight of the filter tube. Difficulty has been experienced in weighing fritted glass filter tubes to constant weight which previously had been cleaned with sulfuric acid-dichromate cleaning mixture. Upon subsequent treatment with nitric acid the same filter tubes became constant in weight.

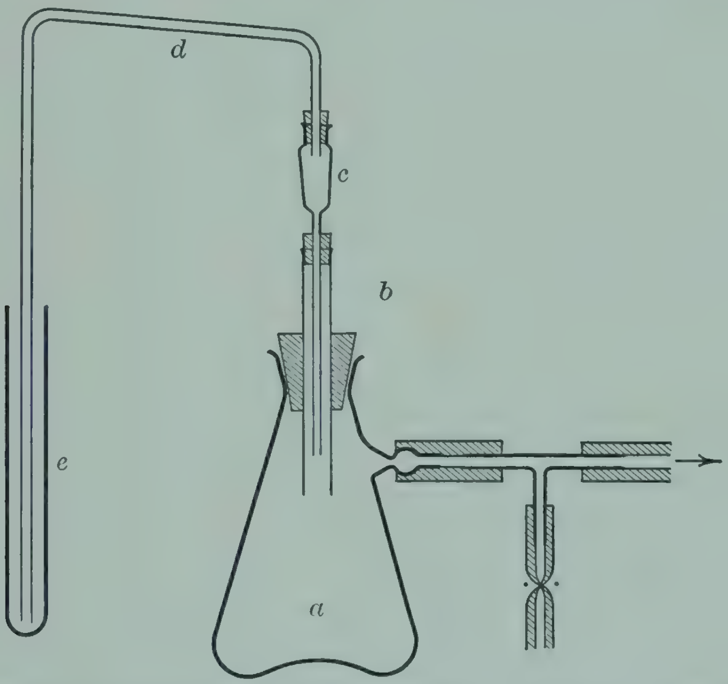


Fig. 38. Filtration Apparatus. *a*, Suction Flask; *b*, Adapter; *c*, Filter Tube; *d*, Siphon; *e*, Pressure Tube.

Filtration Apparatus (Fig. 38). The apparatus consists of a 500-ml. suction flask provided with a tight-fitting rubber stopper, through which is inserted a glass adapter 8 cm. long and 8 mm. in diameter. This adapter is so adjusted that it extends several centimeters above the rubber stopper of the suction flask; its upper opening is provided with a perforated rubber stopper through which the filter is inserted during the filtration. Attached to the side arm of the suction flask is a T-tube carrying a screw clamp for the regulation of pressure.

The solution and the precipitate are siphoned from the pressure tube with the aid of a siphon, which is a glass tube of 2-mm. inner diameter, bent as shown in the illustration. The long vertical arm is 25 cm. long and is bent at an angle of about 80 degrees; then the siphon extends for a length of approximately 10 cm., where it is bent at an angle of about 100 degrees, so that the short arm, which is 6 cm. long, is parallel to the other.

Wash Bottles of the type shown in Fig. 6 are used as containers for the wash liquids such as distilled water and alcohol.

Reagents

All initial oxidizing and precipitating agents, as well as the wash liquids employed, must be free of halogen. The nitric acid, ethyl alcohol, and distilled water are tested for halogen by adding 5 drops of concentrated nitric acid and the same volume of silver nitrate solution to 5 ml. of the reagent and heating it on the steam bath for ten minutes. The reagent is considered halogen-free if, after the above treatment, no precipitation or even opalescence is discernible.

Concentrated Nitric Acid (sp. gr.: 1.42). It is redistilled in an all-glass apparatus over silver nitrate in vacuum.

Silver Nitrate. C.P. crystals.

Potassium Iodide Solution (30%). Thirty grams of C.P. potassium iodide is dissolved in enough distilled water to bring the total volume to 100 ml. The solution must be repeatedly filtered to ensure complete freedom from any suspended foreign matter such as dust particles. The solution is kept in a brown glass-stoppered bottle.

Ethyl Alcohol (95%). Redistilled over silver nitrate.

Distilled Water. Redistilled over silver nitrate.

Procedure

Preparation and Weighing of the Sample. A 5-mg. sample is usually taken for the chlorine, 7-mg. for the bromine, and 10-mg. for the iodine determination. With an ordinary analytical balance of proper sensitivity and precision, about twice the above amounts of sample are taken, but at least 20 mg. for the iodine determination.[75] The amount of silver halide formed often weighs less than the original substance; the amount of substance used for the analysis may then exceed the above limits.

Filling the Pressure Tube. The sample is introduced into the pressure tube in the usual manner, that is, dry solids are introduced with the weighing tube (p. 44), low-boiling liquids (up to 200° C.) by means of a weighing pipet (p. 46), high-boiling liquids (above 200° C.) and semi-solids by employing capillaries which are open at both ends (p. 45). Then a crystal of silver nitrate, not more than a 10% excess over the required amount (about 10 mg. for chlorine and 15 mg. for bromine and iodine), is dropped into the pressure tube. After the silver nitrate, 0.3 ml. (5 or 6 drops) of concentrated nitric acid is added with a dropping pipet in such a manner that it rinses the lower half of the wall of the pressure tube, but wetting of the upper section, which is to be sealed off, is avoided.

If the substance is volatile, or if the halogen in the substance is so reactive that decomposition ensues at room temperature upon contact with the nitric acid, the substance should be introduced into the pressure tube in either a capillary or capillary pipet in such a manner that the substance is *above* the nitric acid until the pressure tube has been sealed. This is most easily accomplished by moistening the wall of the pressure tube, or the outside of the capillary containing the substance, with a drop of nitric acid and thus causing the capillary to adhere to the wall of the pressure tube. By means of a glass rod the capillary is pushed down to within several millimeters of the nitric acid already present in the pressure tube. For reactive solids, capillaries open at both ends, with the substance placed in the center, are used. After the pressure tube has been sealed the substance is brought in contact with the oxidizing agent through proper tilting of the pressure tube.

SEALING THE PRESSURE TUBE

The tube may be sealed either with an ordinary Tirrill gas burner or with a blast lamp.

Sealing with the Tirrill Burner.[102] The pressure tube is held in both hands in a slightly inclined position and is preheated with a luminous flame, while it is constantly rotated, until deposits of carbon are obtained at the part of the tube where it is to be sealed off, that is, about 6 cm. from the open end. Then this area (Fig. 39*a*) is heated with a strong hissing flame, while the tube is slowly rotated. When the glass has softened sufficiently so that the wall of the pressure tube begins to collapse, it is removed from the flame and, while still being turned, drawn out until it forms a constricted region about 2 cm. long (*b*). Then the flame is applied to the base of the constriction and the heating continued, while the tube is turned, until its wall collapses. After the wall of the pressure tube has thickened to form a solid glass rod, the tube is removed from the flame and drawn out as shown in (*c*). It is sealed at the top to a point (*d*) by holding its tip in the outer cone of the flame, and then it is annealed in the luminous flame of the burner.

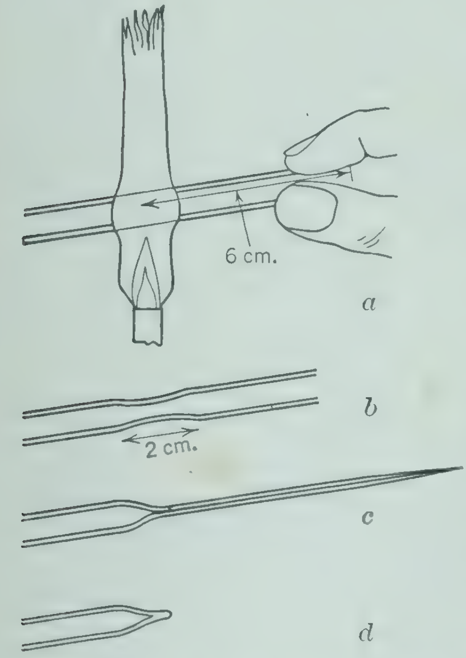


Fig. 39. Sealing of the Pressure Tube with a Tirrill Burner.

Sealing with the Blast Lamp. In sealing the pressure tube with the blast lamp it is advisable to fuse on a short piece of the same kind of tubing, which is made to serve as a handle. The pressure tube is heated over an area of 3 cm. at a point about 6 cm. from the junction, first with a luminous flame and then with a strong hissing flame, while it is constantly rotated, but without pulling, until its wall thickens to a capillary. Then the tube is removed from the flame and drawn out; the extended capillary is heated again with a small but strong flame at its base until the capillary collapses to form a solid glass rod. This end is drawn out once more and after being cut with the flame it is fire-polished; the formation of a curved tip should be avoided.

Digestion. The sealed, but already cool, pressure tube is placed in the furnace and heated for one hour between 280 and 300° C., which is usually sufficient for most halogen compounds.

Opening of the Pressure Tube. At the end of the digestion period the tube is allowed to cool to room temperature in the furnace and then is pushed out with a glass rod, so that the sealed tip protrudes about 1 cm. beyond the end of the furnace. The tip is cautiously heated by passing over it the luminous flame of a Tirrill burner until any liquid caught in the tip is driven down into the tube. Next, the tip is heated directly below the point of taper with a small flame until the glass softens sufficiently to permit the internal pressure of the tube to blow it open (Fig. 40*a*, *b*). After release of the pressure, a groove is filed around the tube with a sharp triangle file, or cut with a small Griffin glass-cutting wheel, somewhat below the point of taper. The area of the groove is moistened with water and then, with the tube held in an inclined position, the molten tip of a thin glass rod is pressed against the groove; this procedure is repeated if necessary until a crack encircles the tube. The pressure tube, while held in a towel, is pulled apart and the edge fire-polished. If it is pulled apart rather than broken by exerting angular force, glass splinters will rarely enter the tube.

Filtering the Precipitate. The clean filter tube is inserted in the adapter, and the short arm of the siphon, which is provided with a smooth rubber stopper, is placed on the filter tube. The contents of the pressure tube are diluted with 2 to 3 ml. of halogen-free distilled water, or with no more than enough to fill two-thirds of the tube; the tube is placed in a 250-ml. beaker half filled with water which is then boiled for several minutes. The hot water in the beaker is replaced by cold water and the pressure tube is placed in it to cool. When cool, the pressure tube is placed under the siphon of the filtration apparatus. The precipitate is stirred with the long arm of the siphon and lumps of the precipitate may be crushed at the same time. Suction is then applied and the precipitate and wash liquid are siphoned over, but the speed of filtration is so regulated with a screw clamp on the vertical arm of the T-tube between the pressure tubing and the suction flask that the liquid drops on the filter mat, or sintered glass plate, at a rate of 1 to 2 drops per second. The bulb of the filter tube should never be filled completely during the filtration; before this occurs the long arm of the siphon is raised above the meniscus of the liquid in the pressure tube to allow the liquid in the bulb of the filter tube to be drawn off.

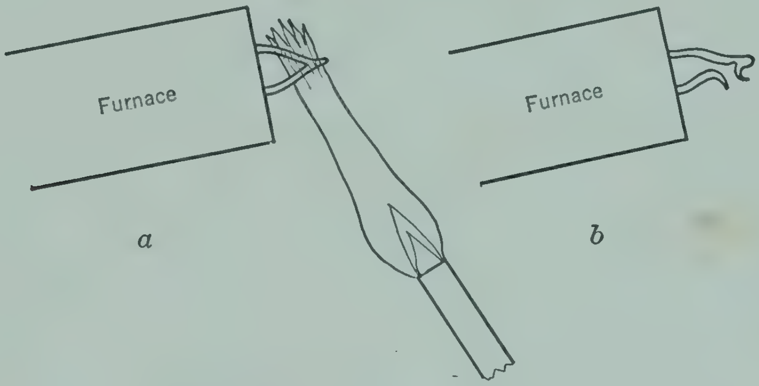


Fig. 40. Releasing the Pressure.

After the liquid and whatever precipitate was carried over have been siphoned off, the suction is broken by opening the screw clamp; then the tube is filled two-thirds full with distilled water by spraying the wash liquid over the inner wall of the pressure tube while it is being rotated. Suction is again applied and the liquid drawn off; at this point of the procedure it is advisable to admit air bubbles into the siphon by repeatedly raising the siphon above the liquid in the pressure tube, because these air bubbles act as a scrubber and very effectively remove particles of precipitate lodged in the horizontal section of the siphon. This rinsing is repeated with alcohol, then with distilled water, and once more with alcohol; particles of precipitate clinging to the wall of the pressure tube are scraped off with the siphon tube.

After this procedure of rinsing the siphon is removed from the filter tube, but the stopper as well as the short part of the siphon protruding from the stopper are rinsed with alcohol, which is collected in the filter tube and filtered. The suction is then broken and the filter tube is filled to the rim with distilled water which is drawn off; this rinsing is repeated twice with alcohol. After the procedure of rinsing, the filter tube is removed from the filtration apparatus, its outside is wiped dry with a towel, and its orifice is closed with a dust filter; next, the filter tube is connected to a suction pump with a flexible rubber tubing which is intersected by a T-tube for the purpose of regulating the pressure as well as for breaking the suction.

The filter tube is dried by placing the bulb-shaped part in the wide groove of the heating block which previously has been heated to between 110 and 115° C. Barely perceptible suction is applied; after five minutes the shaft of the filter tube is placed in the narrow groove and moved up after two or three minutes to dry the remaining portion of the stem. Then the filter tube is removed from the drying block, laid on the table, and allowed to cool under suction for five minutes, after which the suction is gradually broken by opening the screw clamp on the T-tube. Finally, while being held in a vertical position, the filter tube is wiped once with a moist flannel and three times with a dry chamois. After wiping, the filter tube is placed on a metal rack and left near the balance for fifteen minutes before it is weighed within ±10 micrograms if a microanalytical balance is used, and within ±1 deflection unit if an ordinary analytical balance is employed. The zero reading of the balance must also be determined and should be taken during the fifteen minutes preceding the weighing of the filter tube.

Dissolving the Precipitate. After the filter tube has been weighed it is placed on the filtration apparatus and the bulb is filled with 30% potassium iodide solution with the aid of a medicine dropper; this solution is allowed to run off slowly by itself, or if this is too slow, that is, less than 1 drop per second, under gentle suction. This process is repeated with another portion of the same solution, and if the precipitate has not dissolved by this time the treatment is repeated. Particles of glass should not be mistaken for undissolved silver halide. Finally, the filter tube is washed twice again, but more rapidly, with potassium iodide solution, five times with distilled water, and twice with alcohol. Then it is dried, allowed to cool, wiped, and weighed as before; the difference in weight between the first and second weighings represents the weight of the silver halide obtained.

The above procedure of weighing the filter tube after the filtration of the precipitate, then dissolving the precipitate, and weighing back the filter tube is recommended for students learning the technic of the determination, or if glass splinters have contaminated the precipitate, or when substances are analyzed which required the use of weighing pipets or capillaries; it, therefore, represents a procedure fulfilling all requirements. If, however, substances are analyzed which can be weighed with the weighing tube and the precipitate was not contaminated with glass splinters when the pressure tube was opened, then a somewhat more rapid procedure of weighing can be adopted. In this procedure the filter tube is washed, dried, and weighed while the substance is digested in the furnace, and the weight of the filter tube with the precipitate is the final weight of the first determination; then, without dissolving the precipitate, the next filtration is carried out, and thus the difference between the last two weighings represents the weight of the precipitate of the second determination. This may be continued until an entire series of analyses has been made, thereby effecting the saving of one weighing between each of two analyses. It is advisable, however, to start with a clean filter and to dissolve the precipitate after finishing a series of determinations.

Time:	*Minutes*
Weighing of the sample	10
Preparation, filling and sealing of the pressure tube	15
Digestion	60
Filtration	15
Drying of the filter tube	15
Wiping and weighing of the filter tube with the precipitate	20
Dissolving the precipitate	10
Drying of the filter tube	15
Wiping and reweighing the filter tube	20
Total	180

Calculation:

Log of weight of silver halide,
Plus log of the respective factor,
Plus negative log of weight of sample;
Antilog of total = percentage halogen.

Factors:

Chlorine as silver chloride: 0.2474; log, 39340.
Bromine as silver bromide: 0.4255; log, 62894.
Iodine as silver iodide: 0.5405; log, 73284.

DRY COMBUSTION METHOD

Principle

The substance is combusted in a suitably constructed combustion tube in the presence of oxygen, and the decomposition products are passed over heated platinum contacts to ensure complete oxidation.[31, 50, 71, 80a, 87] The halogen is absorbed in a solution of sodium carbonate containing sodium bisulfite to reduce any chlorate, bromate, or iodate. The absorption reagent which is distributed over the spiral of the combustion tube is transferred to a test tube, and the halogen is precipitated as silver halide by the addition of silver nitrate solution and concentrated nitric acid. The precipitated silver halide is transferred to a filter tube and weighed.

$$(a)\qquad 2RX \xrightarrow[\text{Pt}]{\Delta O_2} X_2 + xCO_2 + xH_2O \quad \text{(oxidation)}$$

$$(b)\qquad X_2 \xrightarrow[NaHSO_3]{+Na_2CO_3} 2NaX \quad \text{(absorption)}$$

$$(c)\qquad NaX + AgNO_3 = AgX + NaNO_3 \quad \text{(precipitation)}$$

Apparatus

Oxygen Tank and Gas Wash Bottle.

Combustion-Spiral Tube (Fig. 41). This tube is made of quartz, Pyrex glass No. 172, or Supremax glass, and is about 64 cm. long and 8 mm. in inner diameter. One end of the tube is drawn out to a capillary of approximately 0.3-mm. inner and 5-mm. outer diameter. A spiral of Pyrex glass approximately 20 cm. long is placed against this capillary and held in position by an indentation in the combustion tube. The mouth of the combustion tube is provided with a perforated rubber stopper in which a capillary is inserted.

Combustion Stand. This is of the same type as that illustrated in Fig. 21.

Platinum Contacts. These are 5 cm. long and 7.5 mm. in diameter, or just somewhat smaller than the bore of the combustion tube. The contacts may vary in form, but those having a starlike cross section are preferable. The ends of the contacts are provided with loops of platinum wire. They are cleaned by boiling in dilute nitric acid and heating in a non-luminous flame; to protect the contacts from dust they are kept in a covered Petri dish.

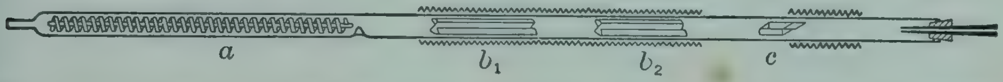


FIG. 41. Halogen Combustion Tube. *a*, Spiral; b_1 and b_2, Platinum Contacts; *c*, Platinum Boat.

Precipitation Tubes. Test tubes of Pyrex glass of 4.5-cm. inner diameter and 20-cm. length are used for the transfer and precipitation of the halogen.

Filter Tubes, Filtration Apparatus, and Wash Bottles. These devices are the same as those used for the wet combustion method (pp. 152-154).

Reagents

All reagents must be halogen-free (see p. 154).

Concentrated Nitric Acid. This is distilled over silver nitrate in vacuum.

Sodium Carbonate Solution. Twenty-five grams of halogen-free sodium carbonate is dissolved in 100 ml. of boiling, halogen-free distilled water. The warm solution is poured into a reagent bottle provided with a rubber stopper and covered with a small beaker.

Perhydrol. Completely halogen-free.

Silver Nitrate Solution (5%). Five grams of C.P. silver nitrate is dissolved in 100 ml. of halogen-free distilled water to which a few drops of nitric acid have been added. It is stored in a brown reagent bottle.

Sodium Bisulfite Solution. This solution is prepared from the halogen-free saturated sodium carbonate solution by slowly introducing halogen-free sulfur dioxide with cooling, because otherwise appreciable quantities of thiosulfate are formed which cause the separation of sulfur on acidification. The sulfur dioxide is generated in a small generator by slowly adding concentrated sulfuric acid to sodium bisulfite and passing the liberated sulfur dioxide through a tube containing glass wool, which is moistened with halogen-free saturated sodium carbonate solution, before it is passed into the cooled sodium carbonate solution. The sodium bisulfite solution so obtained is stored in ampules of approximately 25-ml. capacity drawn out to a long capillary. Before use the capillary is opened near its top and the solution expelled from the ampule drop by drop; then the capillary is sealed off again.

This solution should conform to the following test: Twenty milliliters, made alkaline with halogen-free sodium carbonate solution to which 3 or 4 drops of perhydrol have been added, is warmed on a steam bath for five minutes. After cooling, a mixture of 1 to 2 ml. of halogen-free nitric acid and 0.5 ml. of 5% silver nitrate solution is added. The reaction mixture is then heated for an additional ten minutes on a steam bath, and the solution can be considered free from halogen when no precipitation or turbidity occurs.

Ethyl Alcohol (95%).

Distilled Water.

Procedure

Preparation and Filling of the Combustion Tube. Before every determination the combustion tube must be cleaned with hot cleaning solution which is siphoned up through the capillary end and allowed to remain in the tube for ten minutes. Then the cleaning solution is drained off and the combustion tube is washed successively with tap water, distilled water, and alcohol; after closing the mouth of the tube with a dust filter, the tube is dried on the suction pump with moderate heating. When it has cooled to room temperature, 2 ml. of sodium carbonate solution is mixed with 2 drops of sodium bisulfite solution in a wide test tube and this mixture is siphoned up to the indentation, *but not beyond*, so that the entire spiral is well moistened with the absorption liquid. To prevent saliva from entering the combustion tube in siphoning up the absorption liquid, the mouth of the tube is protected by a dust filter; in all subsequent operations which require siphoning up or expelling of liquid the same precautionary measure is employed. After the spiral has been well moistened with the absorption liquid by rotating the combustion tube, the excess liquid is permitted to drain off and is discarded. The test tube which contained the absorption liquid is placed over the capillary end of the combustion tube where, adequately supported, it remains during the entire combustion. Next, the freshly cleaned platinum contacts are introduced; the first is placed about 8 cm. from the indentation and the second about 5 cm. from the first. The mouth of the combustion tube is closed with a small cotton plug, and the long wire gauze is pushed to within 5 cm. of the indentation, but still should cover completely the area occupied by the platinum contacts. The short wire gauze is placed over the front part of the combustion tube; the cotton plug is removed and replaced by the rubber stopper with the capillary connection.

Combustion. The oxygen necessary for the combustion is obtained from a tank and then passed through a gas wash bottle containing a saturated solution of sodium carbonate. The oxygen enters the combustion tube through the capillary tube at a rate of 5 to 6 ml. per minute, a rate which is determined by counting the number of bubbles rising in the wash bottle.* Next, the long burner under the long wire gauze of the combustion tube is lighted and the platinum contacts heated to a dull red. It is important that the platinum contacts be heated to redness before the sample is introduced into the combustion tube, because otherwise volatile substances may vaporize and pass through the system without being completely oxidized.

The size of the sample is chosen according to the percentage and kind of halogen present in the compound and whether a microanalytical or ordinary analytical balance is used. The substance is weighed in a platinum boat which is then placed within 7 cm. of the platinum contact. The short wire gauze is moved 5 cm. in front of the platinum boat, and the combustion is carried out by gradually advancing a gas burner toward the substance, heating directly under it for ten minutes, and then moving the flame up to the long burner. The entire combustion requires approximately thirty minutes. The combustion may be slowed down when volatile substances are combusted, in order to ensure sufficient contact of the combustion products with the platinum catalysts. After the completion of the combustion both burners are extinguished and the combustion tube is permitted to cool to room temperature in an atmosphere of oxygen. Then the wire gauzes, the platinum boat, and the two platinum contacts are removed. The upper part of the combustion tube is wiped with a clean towel and then is clamped vertically to a stand, without being removed from the wide test tube with which it was covered during the combustion.

Precipitation and Filtration. Eight milliliters of distilled water is poured into the precipitation tube (receiver *A*), 3 or 4 drops of sodium bisulfite solution is added, and the mixture is aspirated into the combustion tube which is protected with a dust filter. The wash liquid should cover the spiral completely and extend 1 cm. beyond the indentation. Then the dust filter is removed, the mouth of the combustion tube closed with the index finger, the tube transferred to a second precipitation tube (receiver *B*), and the wash water is permitted to drain off. Receiver *A* is rinsed with 8 ml. of distilled water which is aspirated into the combustion tube and drained into receiver *B* as before. This procedure is repeated a third time with the same amount of distilled water; in this manner the quantitative transfer of the absorption liquid from the combustion tube to receiver *B* is accomplished. Finally, the inside and the capillary tip of the combustion tube are rinsed with a small amount of distilled water which is also collected in receiver *B*. Two drops of perhydrol are added to the solution in receiver *B* to oxidize any excess of bisulfite, and then the contents of receiver *B* are heated on the steam bath for three to five minutes. After this, 1 ml. of concentrated nitric acid and 2 ml. of 5% silver nitrate solution are added to the contents in the receiver; the receiver is covered with an evaporating dish and heated on the steam bath for fifteen minutes. To facilitate the coagulation of the precipitated silver halide, the receiver is taken from the steam bath at about two-minute intervals and shaken. Finally, the receiver is placed in a beaker of cold water, and, as soon as the supernatant liquid is perfectly clear, the precipitate is ready to be filtered as described on p. 157. Since in this determination no glass splinters can be present in the precipitate, the more rapid procedure of weighing the precipitate (see p. 159) can be employed.

---

* The velocity of the oxygen is determined by connecting the wash bottle to a Mariotte flask and measuring the volume of water displaced in thirty seconds. To obtain the correct volume of oxygen in subsequent determinations, the number of bubbles rising during this interval is counted and then the needle valve of the oxygen tank is simply adjusted to give again the required number of bubbles. It is necessary to make sure that the gas wash bottle is leak-proof, because otherwise the calibration by counting the bubbles is of no value.

Iodine. The determination of iodine by this method requires a slightly modified procedure. A larger sample, about 10 mg., or twice as much if weighed on an ordinary analytical balance, is taken and the combustion is modified to the extent that iodine remaining as a sublimate in front of the spiral must be driven into the absorption liquid by cautiously heating this part of the combustion tube. The transfer of the absorption liquid is carried out as described above, except that 2 drops of sodium bisulfite solution are also added to the 8 ml. of distilled water used for the second washing of the combustion tube and 4 or 5 drops of perhydrol is added to the combined wash water, because of the greater amount of sodium bisulfite solution used. The combined extracts and washings, however, are not heated, but allowed to stand at room temperature for ten minutes. Then 1 ml. of concentrated nitric acid and 2 ml. of 5% silver nitrate solution are added, and the precipitation and filtration are carried out as described above.

Time:	*Minutes*
Weighing of the sample	10
Preparing and filling of the combustion tube	25
Combustion	30
Cooling of the combustion tube (during this period and during the latter part of the combustion the filter tube may be weighed)	20
Filtration	15
Drying of the filter tube	15
Wiping and weighing of the filter tube with the precipitate	20
Total	135

Calculation:

Log of weight of silver halide,
Plus log of the respective factor (p. 297),
Plus negative log of weight of sample;
Antilog of total = percentage of halogen.

FUSION METHOD

BOMB METHOD

Principle

The organic halogen compound is fused with a mixture of potassium nitrate, sodium peroxide, and cane sugar in a microbomb [37, 38] to yield ionizable halogen which is precipitated and weighed as silver halide.

$$(a) \qquad 2RX \xrightarrow[KNO_3;\ Na_2O_2]{\Delta} 2NaX + xCO_2 + xH_2O \quad \text{(fusion)}$$

$$(b) \qquad NaX + AgNO_3 = AgX + NaNO_3 \quad \text{(precipitation)}$$

Apparatus

Bomb (Fig. 42). The bomb employed for the fusion consists of a fusion cup, 2.5 cm. deep and 1.3 cm. in inside diameter; it is made of illium (nickel-chromium alloy). Its wall is 1.5 mm. thick, and it has a lip 3 mm. wide, on which the lid and the lead washer rest. The cup has a rounded base and a small eyelet at the bottom. The lid of the bomb is held securely in position by an arched clamp with a tight-fitting screw.

Precipitation Tubes, Filter Tube, Filtration Apparatus, and Wash Bottles. These are the same as mentioned under "Dry Combustion Method" (p. 161).

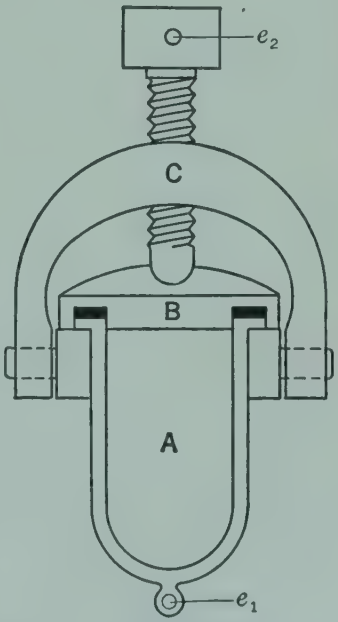


FIG. 42. Bomb; *A*, Cup; *B*, Lid; *C*, Screw Clamp; e_1 and e_2, Eyelets.

Reagents

All reagents must be halogen-free and correspond to the test for the absence of halogen as described on p. 154.

Potassium Nitrate. C.P. crystals.
Sodium Peroxide. C.P.
Concentrated Nitric Acid (sp. gr.: 1.42).
Dilute Nitric Acid (0.1%).
Silver Nitrate Solution (5%).
Hydrazine Sulfate. C.P.
Cane-Sugar, C.P., or *d-Glucose*, C.P.
Ethyl Alcohol (95%).
Distilled Water.

Procedure

Mixing. Three hundred milligrams of a mixture of potassium nitrate and sugar in the proportion of 3 : 1 and 1.5 grams of sodium peroxide are mixed in a glass-stoppered weighing bottle. One-third of this mixture is placed in the cup of the bomb, and an appropriate amount of the substance to be analyzed is added. Then the rest of the mixture is added, the lid of the bomb is screwed tight, and the contents are thoroughly mixed by shaking the bomb. To facilitate complete oxidation, efficient mixing is important. After the bomb has been tapped repeatedly so that its contents settle to the bottom, the reaction mixture is ready for the fusion.

Fusion. The bomb is held by the head of the screw clamp either with tongs or by a piece of stout wire passed through the eyelet in the head of the screw and then it is gradually lowered about one-third into the upper part of a small but non-luminous flame of a gas burner; care should be taken to avoid heating too near the lid. The fusion is complete in about ten seconds; the actual fusion can be determined readily because the resulting disturbance within the bomb can be felt. Theoretically the fusion is then completed, but it is advisable to hold the bomb in the flame for an additional five seconds in order to fuse the entire reaction mixture thoroughly. Then the bomb is cooled under the tap.

Precipitation and Filtration. The bomb is first rinsed on the outside with distilled water, then opened, and the inside of the lid is washed with hot distilled water; the washings are collected in a precipitation tube. Next, the cup is placed in the precipitation tube and enough hot distilled water is added to cover the bomb, which is agitated to effect complete solution of the fusion mixture. After the fusion mixture has completely dissolved, the cup is lifted up with a heavy platinum wire and thoroughly rinsed with hot distilled water. Then the cup is picked up with platinum-tipped forceps at the eyelet and the inside of the cup is also washed with hot distilled water, which is added to the original solution.

Chlorine. This solution is cooled in an ice bath and acidified with concentrated nitric acid. The acidified solution is filtered into another precipitation tube, and the filtrate is treated with 1 ml. of 5% silver nitrate solution; it is reheated on a steam bath and the precipitate is filtered as described on p. 157.

Bromine and Iodine. The combined extracts and washings are neutralized to a slight pink with nitric acid, phenolphthalein being used as the indicator. Then 100 mg. of hydrazine sulfate is added and the mixture is heated on a steam bath for fifteen minutes. After the mixture has been filtered into another precipitation tube, it is acidified with 0.5 ml. of concentrated nitric acid; 1 ml. of 5% silver nitrate solution is added; then it is reheated on the steam bath and the precipitate filtered as described on p. 157.

It is expedient to weigh the required quantities of fusion mixture and hydrazine sulfate and to place these reagents in a spoon of appropriate capacity, or in a small test tube, and mark the level of the reagents. Since the amount of reagent used need be only approximate, later quantities can be measured in this manner instead of being weighed.

Time:

	Minutes
Weighing of the sample	10
Preparation and filling of the bomb	10
Fusion	5
Extraction and precipitation	20
Wiping and weighing of the filter tube	20
Filtration	15
Drying of the filter tube	15
Wiping and weighing of the filter tube with the precipitate	20
Total	115

Calculation:

Log of weight of silver halide,
Plus log of the respective factor (p. 297),
Plus negative log of weight of sample;
Antilog of total = percentage halogen.

TITRIMETRIC METHODS

CHLORINE AND BROMINE

Principle

The organic substance containing chlorine or bromine is placed in a reaction flask and decomposed with concentrated sulfuric acid in the presence of a mixture of potassium dichromate and silver dichromate at a temperature of 115 to 125° C.[115] The combustion is carried out in oxygen, and the halogen is driven into a receiver filled with an excess of 0.01 N sodium hydroxide solution, to which 1 ml. of 30% hydrogen peroxide has been added.

$$(a)\quad 2RX \xrightarrow[H_2SO_4;\ K_2Cr_2O_7;\ Ag_2Cr_2O_7]{O_2} X_2 + xCO_2 + xH_2O \quad \text{(oxidation)}$$

$$(b)\quad X_2 + 2NaOH + H_2O_2 = 2NaX + O_2 + 2H_2O \quad \text{(absorption)}$$

The excess of 0.01 N sodium hydroxide is titrated with 0.01 N acid, and from the amount of alkali used the percentage of chlorine or bromine is calculated. The presence of iodine does not interfere, because it is oxidized to iodate; volatile substances, or substances containing as additional elements nitrogen or sulfur, however, cannot be analyzed by this method.

Apparatus

Oxygen Tank and Gas Wash Bottle. The same oxygen tank and wash bottle as described on p. 162 is used; the flow of oxygen, 8 ml. per minute, is standardized with the aid of a Mariotte flask.

Combustion Apparatus (Fig. 43). The apparatus, made of Pyrex glass, consists of the inlet tube (a), the dropping funnel (b), the introduction tube (c), the reaction flask (d), the delivery tube (e), the absorption buret (f), and the spiral (g). The dropping funnel has a capacity of about 5 ml.; it is 4 cm. long and 2 cm. in diameter. It ends in a stopcock, to which is sealed the introduction tube; this tube is 11 cm. long and has a 4-mm. outer and 2-mm. inner diameter. The reaction flask is 9.5 cm. long over all and has a heart-shaped bulb of 5-ml. capacity at the bottom. Its neck is 7.5 cm. long, 1.2 cm. in diameter, and ends in an interchangeable ground-glass joint. The delivery tube is sealed to the dropping funnel below the stopcock; it extends horizontally for 8 cm. and then descends vertically for 27 cm. The horizontal arm carries a split cylindrical cork for the attachment of the apparatus to a stand; a small glass knob is fused to the vertical arm of the delivery tube 1 cm. above its tip to hold the spiral in place. The absorption buret is 30 cm. long over all and has a cylindrical funnel 6 cm. long and 3.5 cm. in diameter, which narrows down to a buret 17 cm. long and 1.3 cm. in diameter. A stopcock is sealed to the end of the buret at an oblique angle, and below the stopcock is an outlet tube of 4-cm. length and 1-mm. bore.

Heating Block. The apparatus is provided with either a heating block or a suitable metal or oil bath. The heating block consists of a piece of aluminum having a wide enough opening for the insertion of the reaction flask and a smaller one for the thermometer; it is heated by a microburner. The oil bath may be of any convenient shape and is also heated by a microburner.

Titration Equipment. Titration flasks, burets, steaming apparatus, as described under " Standard Solutions " (p. 51), are required.

Reagents

Concentrated Sulfuric Acid (sp. gr.: 1.84). This should be taken from a fresh bottle.

Potassium and Silver Dichromate. This mixture consists of equal parts of potassium dichromate (C.P.) and silver dichromate. The silver dichromate is prepared [5] by boiling 10 grams of silver nitrate and 6 grams of chromic acid in 1 liter of distilled water until the reagents have completely dissolved. The solution is filtered while still hot and is left standing over night. The silver dichromate which crystallizes out in brown crystals is filtered off on a Büchner funnel, washed repeat-

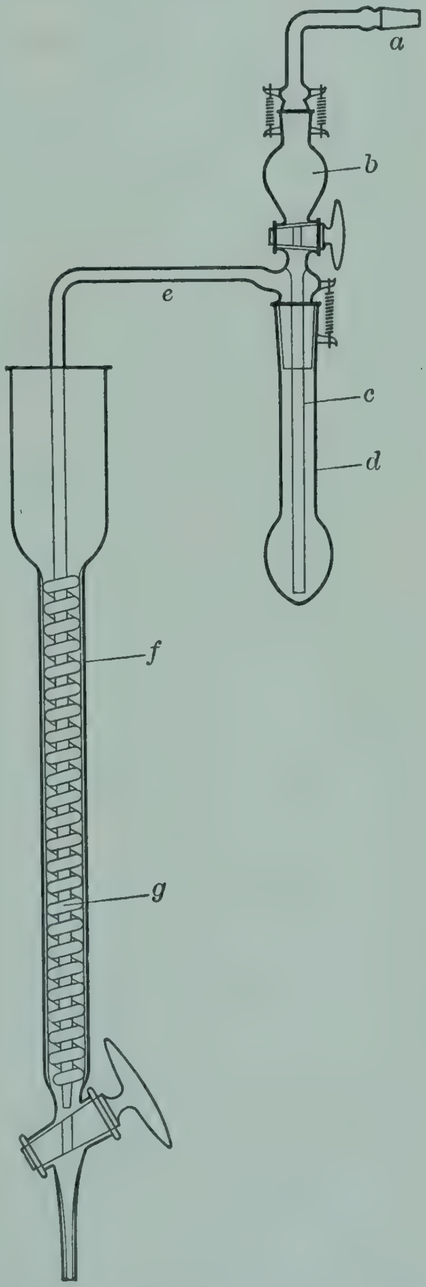


FIG. 43. Apparatus for the Alkalimetric Determination of Chlorine and Bromine. Explanation in Text.

edly with distilled water, and dried in the desiccator over phosphorus pentoxide. It is powdered, mixed with potassium dichromate, and stored in a brown ground-glass stoppered reagent bottle.

Perhydrol (30% hydrogen peroxide). Even the guaranteed acid-free perhydrol shows a slight acidic reaction, and consequently this acidity has to be determined for each fresh bottle. The 0.01 *N* sodium hydroxide solution used for the neutralization of 1 ml. of perhydrol, the *blank*, must be deducted from the total 0.01 *N* sodium hydroxide solution used for a regular determination. This titration is carried out by measuring 1 ml. of perhydrol with a delivery pipet into a 50-ml. Erlenmeyer flask, adding 5 ml. of distilled water, and boiling the solution for a few seconds. A trace of methyl red indicator solution is added and the solution titrated with 0.01 *N* sodium hydroxide solution to a canary-yellow coloration.

0.01 *N Acid Solution.*

0.01 *N Sodium Hydroxide Solution.*

Methyl Red Indicator Solution.

The preparation and standardization of the 0.01 *N* solutions, as well as the preparation of the methyl red indicator solution, are described under " Standard Solutions " on pp. 54-56.

Procedure

Preparation of the Apparatus. Before a series of analyses, especially after having been idle for some time, all parts of the apparatus are cleaned with warm cleaning solution; exhaustively rinsed with tap water, distilled water, and ethyl alcohol; and dried in the drying oven at 120° C. The stopcock of the absorption buret, which is removed during the cleaning of the apparatus, is sparingly but nevertheless sufficiently greased to make the barrel transparent. The perfectly dry apparatus, with the exception of the reaction flask and the absorption buret, is clamped to a stand, and the heating block or oil bath is heated to 115 to 125° C. A volume of 8 ml. of 0.01 *N* sodium hydroxide solution, accurately measured, is run from the microburet into a steamed-out 125-ml. Erlenmeyer flask, and 1 ml. of perhydrol is added to the alkali with a 1-ml. delivery pipet. This solution is poured into the absorption buret, which is then attached to the apparatus so that the tip of the delivery tube extends within 5 mm. of the stopcock; the buret is held in place with the aid of a loose clamp which allows free rotation of the buret. The Erlenmeyer flask is placed under the outlet tube of the stopcock, because it is used again to collect the absorption liquid after the combustion. The sample is weighed in the weighing tube or.

if a liquid, in the weighing capillary and transferred to a clean, dry reaction flask. About 0.5 gram of the dichromate mixture is added to the substance with a small spoon of known capacity, so that the amount of reagent, which need be only approximate, does not have to be weighed. Then the ground-glass joint of the reaction flask is lubricated with a few drops of sulfuric acid, after which it is attached to the apparatus and fastened with two wire springs. The stopcock of the dropping funnel is also lubricated with a little sulfuric acid and turned shut; 2 ml. of concentrated sulfuric acid is placed in the dropping funnel with a delivery pipet, and the inlet tube, which connects the apparatus to the oxygen tank, is inserted in the dropping funnel. Then the oxygen, which previously has been regulated to a velocity of 7 to 8 ml. per minute, is turned on and the stopcock gradually opened, the sulfuric acid being forced thereby slowly down into the reaction flask.

Heating and Titration. After the sulfuric acid has run into the reaction vessel and the initial reaction has subsided, as evidenced by a regular rise of bubbles from the delivery tube in the absorption buret, the heating block or oil bath is raised high enough to cover completely the bulb of the reaction flask, which is then heated to 115–125° C. for thirty minutes. At the end of this period the stopcock above the reaction flask is closed and the inlet tube disconnected from the dropping funnel; then the stopcock below the absorption buret is opened and the absorption liquid drained into the Erlenmeyer flask. The stopcock is again closed and the absorption buret rinsed, while being rotated, with 4 to 5 ml. of distilled water. Before the wash liquid is drained into the Erlenmeyer flask, the apparatus is again connected to the oxygen tank, the stopcock below the dropping funnel is opened, and oxygen is bubbled through the system for about twenty seconds to expel any absorption liquid remaining inside of the delivery tube. The rinsing of the absorption buret is repeated twice in precisely the same manner. A trace of methyl red indicator solution is added to the solution in the Erlenmeyer flask and the solution titrated with 0.01 N acid to a distinct red; then it is boiled for about twenty seconds to expel carbon dioxide. Another trace of indicator solution is added and the solution is titrated back with 0.01 N sodium hydroxide solution to a canary-yellow coloration. Should the solution turn yellow upon boiling, more acid is added and the boiling repeated.

The apparatus must be rinsed with distilled water and ethyl alcohol and dried before it is used for the next analysis. The substance is most conveniently weighed during the thirty minutes of the combustion, because the apparatus, aside from an occasional check of the temperature and velocity of the oxygen, needs no further supervision.

Time:

	Minutes
Weighing of the sample	10
Preparation and filling of the apparatus	20
Combustion	30
Rinsing the absorption buret	5
Titration	10
Total	75

Calculation:

Log of ml. 0.01 *N* sodium hydroxide solution,
Plus log of atomic weight of the respective halogen,
Plus negative log of weight of sample;
Antilog of total = percentage halogen.

Factors:

Chlorine: 35,457; log, 54970.
Bromine: 79,916; log, 90263.

IODINE

Principle

The organic iodine compound is combusted catalytically in an atmosphere of oxygen [66, 108, 109] to yield iodine, carbon dioxide, and water. The iodine is absorbed in a suitable medium and is oxidized with bromine to form iodic acid which is determined iodometrically.

The steps involved are the following:

$$(a)\quad 2RI \xrightarrow[Pt,\ O_2]{\Delta} I_2 + xCO_2 + xH_2O \quad \text{(combustion)}$$

$$(b)\quad I_2 + Br_2 = 2IBr$$

$$(c)\quad IBr + 2Br_2 + 3H_2O = HIO_3 + 5HBr$$

$$(d)\quad HIO_3 + 5HI = 3I_2 + 3H_2O$$

$$(e)\quad 3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6 \quad \text{(titration)}$$

Apparatus

Oxygen Tank and Gas Wash Bottle.
Combustion Tube.
Combustion Stand.
Platinum Contacts.
Precipitation Tubes.

These five items are the same as described under "Dry Combustion Method" on p. 160.

Titration equipment is the same as described under "Standard Solutions" on p. 51.

Reagents

Sodium Hydroxide Solution (5%). Five grams of c.p. sodium hydroxide is dissolved in 100 ml. of distilled water.

Sodium Acetate Solution (20%). This is prepared by dissolving 20 grams of sodium acetate ($CH_3COONa + 3H_2O$) in 100 ml. of distilled water.

Solution of Sodium Acetate in Glacial Acetic Acid (10%). Ten grams of sodium acetate ($CH_3COONa + 3H_2O$) is dissolved in 100 grams of glacial acetic acid.

Bromine. Free from iodine; it is kept in a dropping bottle.

Formic Acid (80 to 100%). This is kept in a dropping bottle.

Potassium Iodide Solution (free from iodate). Ten per cent aqueous solution.

Sulfuric Acid (2 *N*).

0.01 *N Sodium Thiosulfate Solution.*

Starch Indicator Solution.

Methyl Red Indicator Solution.

The standard solutions and the indicator solutions are the same as described under "Standard Solutions" on pp. 53-57.

Distilled Water. It is advisable to redistil the distilled water which is used as wash liquid or for the preparation of the various reagents.

Procedure

Combustion. The spiral combustion tube is cleaned and dried as described on p. 162. For the absorption of iodine, 5 ml. of 5% sodium hydroxide solution is aspirated from a precipitation tube up to the end of the spiral; then the excess absorption liquid is permitted to drain back into the tube and is discarded. The empty tube is then placed over the outlet of the combustion tube. Next, the platinum contacts are inserted in the combustion tube, the pieces of wire gauze are placed over it, and the long burner is lighted. After the platinum contacts have been heated to a dull red, the substance, which is weighed in a platinum boat if a solid, or in a capillary if a liquid, is introduced, and finally the combustion tube is connected to the oxygen tank. The combustion is carried out as described on p. 162, except that the oxygen passing through the system is adjusted to a velocity of 3 ml. per minute. Caution must be exercised during the combustion so that no iodine sublimes behind the movable burner; should this occur, but provided that it is not too close to the rubber stopper, then the sublimate is driven back toward the platinum contacts by appropriate heating. After completion of the combustion the section of the combustion tube between the long burner and the beginning of the spiral must be examined for deposits of iodine, which, if present, are driven into the absorption liquid by cautious heating with the gas burner. The combustion tube is permitted to cool while oxygen is passed through at the normal rate. During this period the necessary preparations for the titration are made.

Transfer and Titration. Measured with a delivery pipet, 5 ml. of 20% sodium acetate solution is placed in a 125-ml. Erlenmeyer flask (receiver *B*) having a ground-glass stopper. For the rinsing of the combustion tube 4 ml. of 10% sodium acetate solution in glacial acetic acid is placed in a test tube and 2 or 3 drops of bromine is added. Then the wire gauzes, platinum boat, and platinum contacts are removed from the combustion tube, which is taken from the stand and placed in an upright position without removing it from the precipitation tube that covered it during the combustion. The prepared reagent of 10% sodium acetate solution and bromine in glacial acetic acid is poured into this precipitation tube (receiver *A*) and aspirated 1 to 2 cm. beyond the spiral of the combustion tube. With the mouth of the combustion tube closed with the index finger, the capillary end is placed in the Erlenmeyer flask (receiver *B*) containing the 20% aqueous sodium acetate solution; the combustion tube is clamped vertically to a stand, and the aspirated solution is allowed to drain into the Erlenmeyer flask. About 6 ml. of distilled water is placed in the precipitation tube (receiver *A*), aspirated into the combustion tube, and again transferred to the same Erlenmeyer flask. With the aid of a graduated wash cylinder the combustion tube is rinsed, while being rotated, with two more 6-ml. portions of distilled water, without removing the tube from the Erlenmeyer flask.

To destroy the excess of bromine 2 or 3 drops of formic acid is run down the wall of the Erlenmeyer flask and its contents are shaken. When the solution is decolorized, it is tested for free bromine by adding a trace of methyl red indicator solution. If the indicator is decolorized, free bromine is still present and another drop of formic acid is added. If the solution remains pink, 2 ml. of 10% potassium iodide solution and 5 ml. of 2 *N* sulfuric acid are added and the solution is left standing in the stoppered Erlenmeyer flask for five minutes. Then the iodine is quickly titrated with 0.01 *N* sodium thiosulfate solution to a faint yellow color; 4 to 6 drops of starch indicator solution is added, and the solution is finally titrated to a slight pink coloration.

Time:

	Minutes
Weighing of the sample	10
Preparation and filling of the combustion tube	25
Combustion	30
Cooling of the combustion tube (during this time the reagents for the titration are prepared)	20
Transfer of the absorption liquid	10
Titration	15
Total	110

Calculation:

Log of ml. 0.01 N sodium thiosulfate solution,
Plus log of factor (32562),
Plus negative log of weight of sample;
Antilog of total = percentage iodine.

Remarks

Reviews and Reports. Existing methods for the determination of halogens in organic compounds have been extensively reviewed by F. Hernler and R. Pfenningberger (fluorine and chlorine, 399 papers);[48] T. Leipert (bromine, 122 papers;[66a] iodine, 155 papers[66b]); Th. v. Fellenberg (iodine, 42 papers);[43] and G. Lunde and co-workers (iodine, 46 papers).[67]

Aside from these reviews numerous investigators have reported on the applications, modifications, difficulties, or deficiencies of existing micro methods for the determination of fluorine,[51, 94] chlorine,[6, 17, 26, 28, 30, 35, 60, 64, 78, 91, 105, 114] bromine,[19, 26] and iodine,[10, 21, 26, 29, 33, 37, 43, 45, 49, 52, 65–67, 69, 82, 84, 95] not only in pure organic compounds but also in biological material,[6, 15, 29, 30, 43, 52, 64, 65, 69, 78, 94] foodstuffs,[91] gasoline,[105] and perfumes.[114]

Wet Combustion Methods. For the quantitative determination of halogen in organic compounds the *Carius Method*[24, 50, 71] possesses numerous advantages which have been successfully incorporated in the respective semi-micro[26] and micro methods.[39, 80a, 87, 102, 104] The method offers a high degree of precision because its reagents are few and are easily purified and, therefore, no blanks have to be taken into consideration. Furthermore, the method involves extremely simple stoichiometric reactions. The objections to the micro-Carius method, such as the danger of explosions, the long heating period, and the possibility of contaminating the reaction product with glass splinters, previously somewhat justified, have been successfully overcome by J. B. Niederl and co-workers[74] through a combination of the early micro-Carius method of F. Emich[39] with the subsequently devised method of F. Pregl[80a, 87] and application of a method of differential weighing to the final reaction product. Through the reduction of the amount of nitric acid employed in the oxidation process the danger of explosions has been practically eliminated, while, by shortening the time of heating to one hour or less, the second objection has been overcome. The third difficulty, the possible contamination of the silver halide precipitate with glass splinters, has been remedied by first weighing the filter tube with the precipitate, then dissolving the precipitate with a saturated potassium iodide solution, followed by reweighing the filter tube, the difference in the weight of the filter tube then giving the weight of the pure silver halide.

The weighing and introduction of the sample contained in a silver foil cup,[56] replacement of the nitric acid by potassium hydroxide and potassium nitrate solutions,[34] volumetric determination of iodine by using mercuric nitrate instead of silver nitrate,[33] special methods of sealing and opening the pressure tubes,[104] and electrically heated furnaces[61] have been described.

The *Dichromate Method* of M. K. Zacherl and H. G. Krainick[115] is based upon the wet macro combustion method of H. Baubigny and G. Chavanne[7] and its subsequent modifications by M. Berend,[10] H. Dieterle,[32] and others.[76, 105] The method involves the use of concentrated sulfuric acid and a mixture of potassium and silver dichromate. It is one of the speediest of the halogen determination methods but has the disadvantage of limited application.[98, 115] Utmost purity of the oxidizing and absorption reagents is essential.

Other wet combustion methods involve the use of *fuming sulfuric acid,*[113] *potassium persulfate,*[101] and *potassium permanganate.*[11, 16, 90, 95] Suitable modifications allow the simultaneous determination of carbon,[85] of arsenic,[27] and of certain metals.[113] The reaction product may be determined gravimetrically,[85, 101, 113] volumetrically,[115] or manometrically.[106]

The macro *Alkaline Reduction Method* as devised by A. Stepanow[97] and others[25, 63, 89, 107] has been adapted with appropriate modifications to the analysis of semi-micro and micro samples of reactive alkyl and aryl halides by W. H. Rauscher[83] and others.[33, 42, 83, 103] Metallic sodium is used in most of these methods, although the use of calcium and lithium[103] also has been suggested. As solvent, low- and high-boiling alcohols,[42, 97] ethanol amine,[83] dioxane,[83] as well as liquid ammonia[25, 107] have been proposed. The halogen is usually determined gravimetrically, but alkalimetry[33] and also titration with the aid of suitable adsorption indicators[41, 42] have been tried successfully.

Dry Combustion Methods. F. Pregl[21, 44, 80a, 87] devised the *Catalytic Oxidation Method* for halogen which was modeled after the semi-micro method of M. Dennstedt.[31] This method was modified by L. T. Hallett[47] for the continuous operation without interruption of the heating process. A quartz absorption tube is sealed vertically to the end of the combustion tube. The combustion gases are bubbled through the absorption liquid which, at the end of the combustion, is rinsed through a stopcock at the bottom of this absorption tube. A detachable spiral tube serving a similar purpose has been described by C. W. Beazley.[9] By dry combustion in an atmosphere of oxygen *with*[21, 31, 44, 47, 52, 70, 80a, 87] or *without*[46, 58, 88, 93] the *platinum catalyzer*, potentiometric[70] and volumetric[52] titration of the halogen, as well as titration involving the use of suitable adsorption indicators,[17, 46, 58, 59, 88, 93] has proved to be applicable.

Hydrogenation Methods. The *Catalytic Hydrogenation Method* of M. Busch[20] and others[14, 54, 86] was first converted to a semi-micro method by H. Ter Meulen and J. Heslinga,[99] and this in turn has been adapted to microchemical work by C. Weygand and A. Werner,[112] K. Bürger,[18] and others.[62, 96, 100] In all these methods the halogen present in the organic compound is converted to the respective hydrogen halide in the presence of hydrogen and a suitable catalyzer, which is then determined gravimetrically,[18, 96] alkalimetrically,[62] or by the Volhard method.[18, 96, 110] Simultaneous determination of nitrogen as ammonia, and of halogen as silver halide, by catalytic hydrogenation has been shown to be feasible by D. Buttescu.[22]

Fusion Method. A. Elek and co-workers[37, 38] successfully transformed the bomb method of S. W. Parr[79] into an efficient micromethod. The reaction product is usually determined *gravimetrically*,[38] but may also be done volumetrically as with iodine,[37] or *colorimetrically*.[77] A semi-micro bomb method has been described by F. E. Beamish.[8] In all these bomb methods purity of the reagents (sodium peroxide, etc.) is absolutely necessary, but difficult to attain.

The Liebig *Calcination Method*[50, 71] has also been converted into a micro method by R. H. Kimball and co-workers.[56] The sample is weighed in a gelatin capsule and ignited in a Pyrex ignition tube containing lime. After the ignition the contents of the tube are dissolved in nitric acid and the halogen determined by the Volhard titration method.[110] Wm. M. MacNevin and Wm. H. Baxley[68] use a steel bomb instead of the Pyrex glass ignition tube. Application of the alkali fusion method to the determination of iodine, particularly in biological material, is favored by Th. v. Fellenberg,[43] E. C. Kendall,[55] and numerous others.

Ionizable halogen in organic compounds (quaternary ammonium halides, etc.) may be determined by the method of A. Schlömer,[92] or S. Kamio and H. Sakamoto.[53] The sample is dissolved in water or aqueous alcohol and is then titrated with 0.01 N silver nitrate solution for chlorine, 0.02 N silver nitrate solution for bromine and iodine, 0.1% bromophenol blue, 0.1% dichlorofluorescein, or 0.2% fluorescein solution respectively, being used as indicators.

Determination of bromine alone in the presence of chlorine[12] and iodine,[111] as well as simultaneous determination of both these elements[72] is possible. A method for the *simultaneous determination of chlorine and bromine* has been devised by L. Moser and R. Miksch.[72] It utilizes the thermal dissociation of ammonium chloride and bromide, and is carried out as follows:

The silver halide precipitate, as obtained by any of the previously described methods, is collected in a Neubauer microcrucible, which is described in the determination of sulfur on p. 194. The crucible is dried in the aluminum block at 150° C., is left to cool on the metal block of the microdesiccator, and weighed. Dry ammonium iodide, free from non-volatile residues, is added to the crucible, in an amount about six times the weight of the precipitate; the crucible is heated at 250 to 300° C., first covered and then open, in a muffle or in the aluminum block, until the ammonium halides have completely volatilized. The crucible is allowed to cool in the microdesiccator and then is weighed. To check the constancy of weight, the heating with ammonium iodide is repeated.

From the weight of the silver chloride and bromide precipitate (first weighing) and the silver iodide formed by the heating with ammonium iodide (second weighing), the chlorine and bromine content of the substance is calculated. The indirect determination of chlorine and bromine when both are present can be carried out with an accuracy of about ±0.5%. Ammonium bromide may be substituted for ammonium iodide.

Modifications in the conventional *iodine* methods involve photometric[40] and also nephelometric[2] determinations. As standards in iodometric titrations, aside from potassium biiodate,[4, 10, 37] ceric sulfate[49] and the sodium salt of acetomercury thymol[13] have been suggested.

The conventional Pregl *filter tube*[23, 80a, 87] has been improved by the introduction of a sintered-glass filter plate which, however, has to be tested for proper retentivity.[74] Substitution of the filter tube by a Gooch crucible of proper dimensions,[36] or by the Schwarz-Bergkampf type of filter beaker,[1] has also been attempted.

LITERATURE

1. Abrahamczik, E., and Blümel, F., *Mikrochim. Acta,* **3**, 185 (1940).
2. Altern, F., and Hille, E., *Mikrochemie,* **19**, 118 (1936).
3. American Platinum Works, Newark, N. J., U. S. A.
4. Andrews, L. W., *J. Am. Chem. Soc.,* **25**, 756 (1903).
5. Autenrieth, W., *Ber.,* **35**, 2057 (1902).
6. Bang, I., *Biochem. Z.,* **49**, 19 (1913); **56**, 158 (1913).
7. Baubigny, H., and Chavanne, G., *Compt. rend.,* **136**, 1197 (1903); **138**, 85 (1904).
8. Beamish, F. E., *Ind. Eng. Chem., Anal. Ed.,* **5**, 348 (1933).
9. Beazley, C. W., *Ind. Eng. Chem., Anal. Ed.,* **11**, 229 (1939).
10. Berend, M., *Biochem. Z.,* **252**, 362 (1934).
11. Berger, H., *J. prakt. Chem.,* **133**, 1 (1932).
12. Berry, A. J., *Analyst,* **64**, 190 (1939).
13. Bordeianu, C. V., and co-workers, *Bul. Soc. Stiinte Fram. România,* **4**, 473 (1939); *Chimie & industrie,* **43**, 458 (1939).
14. Borsche, W., and Heimburger, G., *Ber.,* **48**, 452, 850 (1915).
15. Bratton, A. C., and McClendon, J. F., *Ind. Eng. Chem., Anal. Ed.,* **10**, 600 (1938).
16. Buchholz, J., *Arch. exptl. Path. Pharmakol.,* **81**, 289 (1919).
17. Bullock, B., and Kirk, P. L., *Ind. Eng. Chem., Anal. Ed.,* **7**, 178 (1935).
18. Bürger, K., *Chem. Fabrik,* 1940, p. 218.
19. Busbey, R. L., and Drake, N. L., *Ind. Eng. Chem., Anal. Ed.,* **10**, 390 (1938).
20. Busch, M., *Z. angew. Chem.,* **31**, 232 (1918); **38**, 519 (1925); **47**, 536 (1934); *Ber.,* **49**, 1063 (1916).
21. Butler, A. Q., and Burdett, R. A., *Ind. Eng. Chem., Anal. Ed.,* **11**, 237 (1939).
22. Buttescu, D., *Bul. chim. soc. (România),* **34**, 105 (1931).
23. Canal, F., *Mikrochemie,* **22**, 250 (1937).
24. Carius, L., *Ann.,* **116**, 1 (1860); **136**, 129 (1865); **146**, 301 (1868); *Ber.,* **3**, 697 (1870).
25. Chablay, E., *Ann. chim. phys.,* (9) **1**, 469 (1914).
26. Clark, E. P., *J. Assoc. Official Agr. Chem.,* **16**, 255 (1933).
27. Das Gupta, H. N., *J. Indian Chem. Soc.,* **14**, 358 (1937).
28. Daubney, C. G., *Analyst,* **60**, 29 (1935).
29. Delbridge, T. G., *Am. Chem. J.,* **41**, 397 (1909).
30. Dellavilla, M., and Broun, D., *Bull. soc. chim. biol.,* **9**, 621 (1927).
31. Dennstedt, M., "Anleitung zur vereinfachten Elementaranalyse," V. Meisner's Verlag, Hamburg, 1919.
32. Dieterle, H., *Arch. Pharm.,* **259**, 73 (1921).
33. Doering, H., *Biochem. Z.* **280**, 442 (1935); **290**, 272 (1937); *Ber.,* **70**, 1887 (1937).
34. Dostal, V., *Chem. Listy,* **33**, 78 (1939).
35. Drew, H. D. K., and co-workers, *J. Chem. Soc.,* **1934**, 1787.
36. Dunbar, R. E., *Ind. Eng. Chem., Anal. Ed.,* **9**, 355 (1937).
37. Elek, A., and Harte, R. A., *Ind. Eng. Chem., Anal. Ed.,* **9**, 502 (1937).
38. Elek, A., and Hill, D. W., *J. Am. Chem. Soc.,* **55**, 2550 (1933).
39. Emich, F., and Donau, J., *Monatsh.,* **30**, 745 (1909).
40. Endies, G., and Kaufman, L., *Z. physiol. Chem.,* **243**, 144 (1936).
41. Fajans, K., and Wolff, H., *Z. anorg. allgem. Chem.,* **137**, 221 (1924).

42. Feldman, H. B., and Powell, A. L., *Ind. Eng. Chem., Anal. Ed.*, **11**, 89 (1939).
43. Fellenberg, Th. v., *Mikrochemie*, **7**, 242 (1929) (review, iodine).
44. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Vienna and Leipzig, 1933, p. 103.
45. Goldberg, L. J., *Mikrochemie*, **14**, 161 (1934).
46. Grote, W., and Krekeler, H., *Z. anal. Chem.*, **114**, 321 (1938); **98**, 463 (1934); *Z. angew. Chem.*, **46**, 106 (1933).
47. Hallett, L. T., *Ind. Eng. Chem., Anal. Ed.*, **10**, 111 (1938); "Quantitative Microchemical Analysis," Scott's Standard Methods of Chemical Analysis, D. Van Nostrand Co., New York, N. Y., 1939, pp. 2460–2547.
48. Hernler, F., and Pfenningberger, R., *Mikrochemie*, **25**, 267 (1938) (review, fluorine and chlorine).
49. Hilty, W. W., and Wilson, D. T., *Ind. Eng. Chem., Anal. Ed.*, **11**, 637 (1939).
50. Houben, J., "Die Methoden der organischen Chemie," Verlag G. Thieme, Leipzig, 1925, Vol. I, p. 72.
51. Hubbard, D. M., and Henne, A. L., *J. Am. Chem. Soc.*, **56**, 1078 (1934).
52. Itano, I., and co-workers, *Ber. Ohara Inst. (Japan)*, **8**, 97 (1938).
53. Kamio, S., and Sakamoto, H., *J. Pharm. Soc. Japan*, **58**, 711 (1938).
54. Kelber, C., *Ber.*, **50**, 305 (1917).
55. Kendall, E. C., *J. Am. Chem. Soc.*, **34**, 894 (1912); *J. Biol. Chem.*, **19**, 251 (1914).
56. Kimball, R. H., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **9**, 48 (1937); **10**, 530 (1938).
57. Kirk, P. L., and Dod, K., *Mikrochemie*, **18**, 179 (1935).
58. Koegel, R., *Proc. Pregl Group, Metrop. Microchem. Soc.*, New York, January, 1940.
59. Kolthoff, I. M., *Z. anal. Chem.*, **70**, 395 (1927); **71**, 235 (1927).
60. Korenman, I. M., *Mikrochemie*, **19**, 144 (1936); *Lab. Prakt. (U.S.S.R.)*, **12**, No. 9, 24 (1937); *J. Applied Chem. (U.S.S.R.)*, **10**, 936 (1937).
61. Kuck, J., and Griffel, M., *Ind. Eng. Chem., Anal. Ed.*, **12**, 125 (1940).
62. Lacourt, A., *Compt. rend.*, **203**, 1367 (1936); *Mikrochemie*, **23**, 308 (1938).
63. Landis, Q., and Wichmann, H. J., *Ind. Eng. Chem., Anal. Ed.*, **2**, 394 (1930).
64. Larson, K. O., *Biochem. Z.*, **49**, 479 (1913).
65. Leclerq, L., *J. pharm. belg.*, **17**, 837 (1935); **20**, 233, 253 (1938).
66. Leipert, T., *Mikrochim. Acta*, (*a*) **3**, 147 (1938) (review, bromine); (*b*) **3**, 73 (1938) (review, iodine).
67. Lunde, G., Gloss, K., and Böe, J., *Mikrochemie, Pregl Festschrift*, 1929, p. 272 (review, iodine).
68. MacNevin, Wm. M., and Baxley, Wm. H., *Ind. Eng. Chem., Anal. Ed.*, **12**, 299 (1940).
69. Matthews, N. L., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **10**, 612 (1938).
70. Medinskii, K. B., and Kostrov, I. V., *Zavodskaya Lab.*, **6**, 696 (1937).
71. Meyer, H., "Analyse und Konstitutionsermittlung organischer Verbindungen," J. Springer, Berlin, 1903, p. 138.
72. Moser, L., and Miksch, R., *Mikrochemie, Pregl Festschrift*, 1929, p. 293.
73. Münster, W., *Mikrochemie*, **14**, 23 (1934).
74. Niederl, J. B., Baum, H., McCoy, J. S., and Kuck, J. A., *Ind. Eng. Chem., Anal. Ed.*, **12**, 428 (1940).
75. Niederl, J. B., Niederl, V., Nagel, R. H., and Benedetti-Pichler, A. A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).

76. Nomura, S., and Murai, J., *Bull. soc. chim.*, (4), **35**, 217 (1924).
77. Ochiai, E., and co-workers, *J. Pharm. Soc. Japan*, **57**, 1032 (1937).
78. Oppler, B., *Z. physiol. Chem.*, **70**, 198 (1910).
79. Parr, S. W., *J. Am. Chem. Soc.*, **30**, 764 (1903).
80. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, (*a*) pp. 131–151; (*b*) pp. 179–180.
81. Rappaport, F., and Hohenberg, E., *Mikrochemie*, **14**, 119 (1933).
82. Rappaport, F., and Engelberg, H., *Mikrochemie*, **16**, 1 (1934).
83. Rauscher, W. H., *Ind. Eng. Chem., Anal. Ed.*, **9**, 296, 503 (1937).
84. Reith, J. F., and Dijk, C. P., *Biochem. J.*, **31**, 2128 (1937).
85. Robertson, P. W., *J. Chem. Soc.*, **107**, 902 (1915); **109**, 215 (1916).
86. Rosenmund, K. W., and Zetsche, F., *Ber.*, **51**, 578 (1918).
87. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 94–116.
88. Royer, G. L., *Proc. Pregl Group, Metrop. Microchem. Soc.*, New York, January, 1940.
89. Ruebke, K., *Z. angew. Chem.*, **36**, 156 (1923).
90. Sanchez, J. A., *Analales farm. bioquím. (Buenos Aires)*, **1**, 121 (1930); *Mikrochemie*, **10**, 194 (1931).
91. Scheringa, K., *Chem. Weekblad*, **12**, 702, 749 (1915).
92. Schlömer, A., *Mikrochemie*, **12**, 114 (1933).
93. Schöberl, A., *Z. angew. Chem.*, **50**, 334 (1934).
94. Scott, E. W., and Henne, A. L., *Ind. Eng. Chem., Anal. Ed.*, **7**, 299 (1935).
95. Seeker, A. F., and Mathewson, W. E., *Chem. News*, **103**, 61 (1911).
96. Sloof, A., *Rec. trav. chim.*, **59**, 259 (1940).
97. Stepanow, A., *Ber.*, **39**, 4056 (1906).
98. Stonestreet, G. O., and Wright, G. F., *Can. J. Research*, **18**, B, 246 (1940).
99. Ter Meulen, H., and Heslinga, J., "Neue Methoden der organisch-chemischen Analyse," Akad. Verlagsgesellschaft, Leipzig, 1927.
100. Theilacker, W., and Gessner, E., *Z. angew. Chem.*, **51**, 892 (1938).
101. Thompson, J. J., and Oakdale, V. O., *J. Am. Chem. Soc.*, **52**, 1196 (1930); **55**, 1292 (1933).
102. Tiedcke, C., *Mikrochemie*, **23**, 301 (1938).
103. Tomiček, O., and Petak, K., *Ceskoslov. Lekarnictva*, **17**, 309 (1937).
104. Unterzaucher, J., *Mikrochemie*, **18**, 313 (1935).
105. Utz, F., *Deut. Parfüm. Ztg.*, **8**, 71 (1922).
106. Van Slyke, D. D., and co-workers, *J. Am. Chem. Soc.*, **37**, 1128 (1915); *J. Biol. Chem.*, **21**, 361 (1915); **37**, 551 (1919); **41**, 345 (1920).
107. Vaughn, Th. H., and co-workers, *J. Am. Chem. Soc.*, **55**, 2150, 3453 (1933); **56**, 2064 (1934); *Ind. Eng. Chem., Anal. Ed.*, **3**, 274 (1931).
108. Vieböck, F., *Ber.*, **65**, 393 (1932); *Mikrochemie*, **11**, 465 (1932).
109. Vieböck, F., and Brecher, C., *Ber.*, **63**, 3207 (1930); **65**, 493 (1932).
110. Volhard, J., *Ann.*, **190**, 23 (1878); *J. prakt. Chem.*, **117**, 217 (1874).
111. Weszelszky, J. V., *Z. anal. Chem.*, **39**, 81 (1900).
112. Weygand, C., and Werner, A., *Mikrochemie*, **26**, 177 (1939).
113. Willard, H. H., and co-workers, *J. Am. Chem. Soc.*, **52**, 1893 (1930); **60**, 2869 (1938).
114. Wirth, C., and Stross, M. J., *Ind. Eng. Chem., Anal. Ed.*, **5**, 87 (1933).
115. Zacherl, M. K., and Krainich, H. G., *Mikrochemie*, **11**, 61 (1932).

VII. DETERMINATION OF SULFUR

GRAVIMETRIC WET COMBUSTION (CARIUS) METHOD

Principle

The organic sulfur compound is oxidized by *wet combustion* in the presence of concentrated nitric acid and sodium chloride at a temperature between 280 to 300° C.[10, 15, 40, 44, 46, 59] The sulfur present is thus oxidized to sulfuric acid, which in turn reacts with the sodium chloride to yield sodium sulfate. After dilution and filtration the sodium sulfate is converted to barium sulfate, which is filtered by inverted filtration. After ignition the barium sulfate is weighed with both crucible and immersion filter.

(*a*) Organic S-compound $\xrightarrow[HNO_3+NaCl]{\Delta}$ $Na_2SO_4+xCO_2+xH_2O$ (oxidation)

(*b*) $Na_2SO_4 + BaCl_2 = BaSO_4 + 2NaCl$ (precipitation)

Apparatus

Furnace (Fig. 56),[35, 40, 44, 46] *Pressure Tubes.* These are the same as used in the corresponding *wet combustion method* for the determination of halogen.

Filter Funnel. An ordinary funnel of Pyrex glass having a diameter of 5 cm. at the top and a stem 4 cm. long and 7 to 8 mm. in diameter will serve the purpose.

Evaporation Apparatus (Fig. 44).[40] This apparatus consists of a precipitation or evaporation tube (*a*) of 15-cm. length and 3-cm. outside diameter, having a No. 7 interchangeable ground-glass joint. This joint carries the ground-glass head (*b*), which has a horizontal inlet tube 5 cm. long and 6 mm. in outside diameter; this inlet tube is bent at a right angle where it is sealed to the head and continues vertically for a length of 19 cm., ending in a capillary of 0.5-mm. inner diameter; the outlet tube of this head is 4 cm. long, 6 mm. in outside diameter, and is connected by means of the T-tube (*c*) and a suitably bent glass tubing to the side-arm suction tube (*d*), which serves as a suction flask as well as a trap. Attached to the inlet tube is a capillary tubing in which is placed a tightly compressed plug of glass wool to prevent particles of dust from entering the evaporation tube during the process of evaporation; a wad of glass wool is also inserted into the orifice of the outlet tube. The heating bath (*e*), which is a 1-liter beaker of Pyrex glass containing a saturated salt solution to raise the boiling point of water to 105–110° C. in order to speed up the evaporation, completes the apparatus.

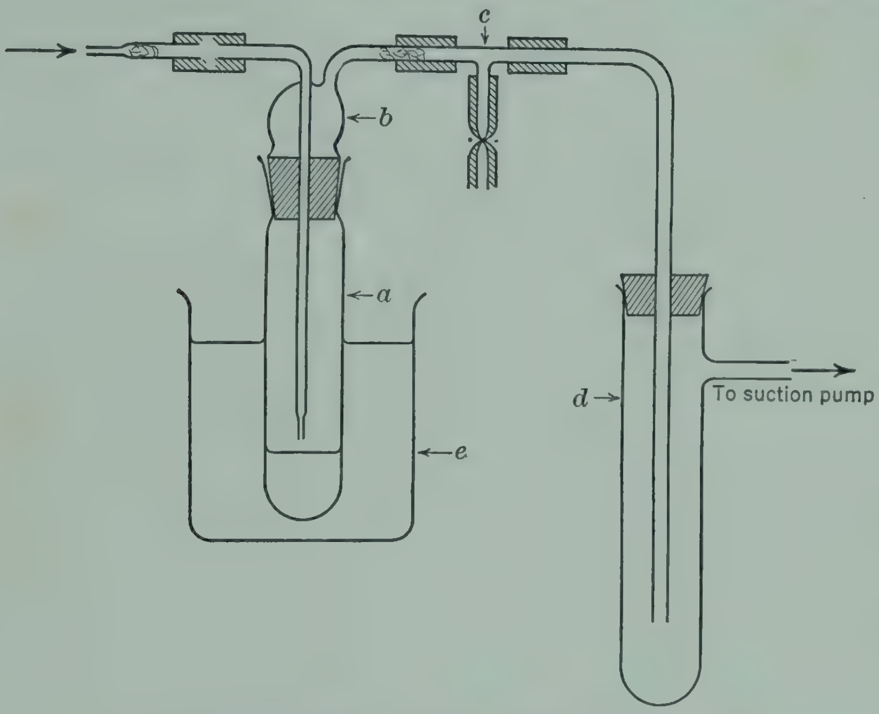


FIG. 44. Evaporation Apparatus. Explanation in Text.

Porcelain Crucible and Immersion Filter (Fig. 45).[15,16, 50] The crucible has a capacity of about 15 ml., is 3.5 cm. high, and has a diameter of 2.8 cm. at the top and 2.2 cm. at the bottom; the inside is glazed black. The immersion filter is also made of porcelain, is 5 cm. long, and has a porous filter plate of 1-cm. diameter; its stem has an outside diameter of 3 mm. and a bore of 1 mm.

Preparation of the Porcelain Crucible and Immersion Filter.[50] To bring the crucible and immersion filter to constant weight, they have to be properly treated. A new crucible is rinsed repeatedly with alcohol and distilled water and then it is wiped inside as well as outside with a clean lint-free cloth; the immersion filter is cleaned by siphoning through 50 ml. of 10% hydrochloric acid solution, which is followed by an equal volume of distilled water which, however, is siphoned through in both directions. A used crucible is cleaned of precipitate from previous analyses by wiping its inside with a cotton wad wound around a toothpick—the knurled iron wire cannot be used because of the possibility of scratching the glazed surface—and then is rinsed repeatedly with distilled water; the outside of the crucible is wiped with a clean lint-free cloth. The immersion filter is freed of precipitate by gently brushing the porous plate with a small brush and then siphoning through distilled water in both directions. Washing with hot concentrated sulfuric acid should be resorted to only in extreme cases. The immersion filter, after being disconnected from the suction pump and its stem wiped with a clean cloth, is placed in the crucible where it remains during the entire procedure of drying, igniting, cooling, and weighing, which is precisely the same before as after an analysis, and is described in detail in the paragraph on drying and igniting. The procedure of washing, drying, igniting, and weighing the crucible and immersion filter is repeated, and the result should agree within ±10 micrograms with the first weighing. They are counterpoised with a ground-glass-stoppered tare bottle containing lead shot.

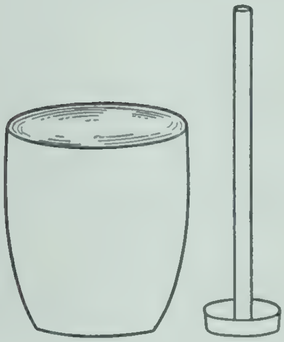


Fig. 45. Crucible and Immersion Filter.

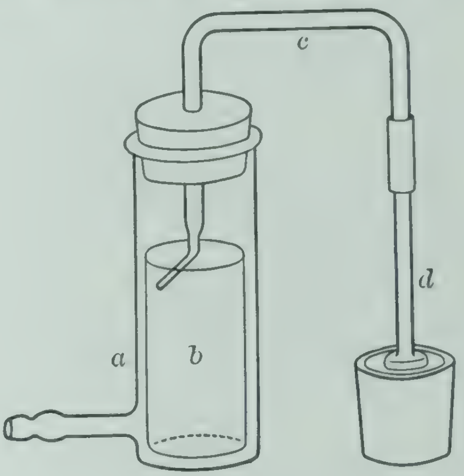


Fig. 46. Apparatus for the Inverted Filtration. Explanation in Text.

Suction Flask (Fig. 46). The suction bottle (*a*) is a flat-bottomed glass tube 11 cm. long and 4 cm. in diameter with a tubulature at the bottom, by which it is connected with a rubber tubing to the suction pump. This rubber tubing is intersected by a glass T-tube, the vertical arm of which is provided with a short rubber tubing carrying a pinch clamp for the regulation of the speed of filtration. A flat-bottomed test tube (*b*), 7 cm. long and 2.5 cm. in diameter, is placed inside the suction bottle to collect the filtrate, so that the filtration may be repeated if necessary. The siphon (*c*) is a capillary glass tubing of 5-mm. outside diameter and 1-mm. bore. The arm which extends through a rubber stopper into the suction bottle is 9 cm. long and ends in an oblique capillary tip; above the rubber stopper it is bent at a right angle to continue horizontally for 7 cm. and then it is bent again to form a vertical arm 5 cm. long, which is also drawn out to a capillary tip having the same outside diameter as the stem of the immersion filter. The immersion filter (*d*) is connected to this tip with a short piece of flexible rubber tubing, while the porous filter plate extends into the crucible.

Reagents

All reagents must be free of sulfur, sulfide, sulfite, and sulfate ions.

Concentrated Nitric Acid (sp. gr.: 1.42).

Concentrated Hydrochloric Acid (sp. gr.: 1.19).

Dilute Hydrochloric Acid (1 : 300).

Sodium Chloride. Reagent quality.

Barium Chloride. C.P. crystals.

Barium Chloride Solution (10%). Prepared by dissolving 10 grams of barium chloride in 100 ml. of distilled water.

Ethyl Alcohol (95%).

Distilled Water.

Procedure

Preparation and Weighing of the Sample. Solids are weighed in the weighing tube; semi-solids such as waxes, fats, and syrups in open capillaries; and liquids in weighing pipets (pp. 44-46). Again, the amount of sample to be taken depends upon the precision of the available balance.

Filling the Pressure Tube. Dry solids are transferred directly into a clean and dry pressure tube. Semi-solids or liquids are introduced in capillaries or capillary pipets; depending upon the reactivity or volatility of the compound it is introduced either before or after the nitric acid. A spatula point of sodium chloride, that is, between 10 and 15 mg., is added to the reaction material.

The *sealing of the pressure tube*, the *digestion process*, and the *opening of the pressure tube* are carried out as described in the corresponding halogen determination method (p. 155), except that the digestion period is extended to two hours.

Transfer of the Reaction Product. The oxidation mixture is diluted with 3 ml. of distilled water, and the resulting solution is filtered through a small funnel, having a small moistened cotton wad as a filter mat, into the precipitation tube of the evaporation apparatus (Fig. 44).

The same part of the pressure tube is again filled with distilled water, which is also filtered through the same funnel and collected in the precipitation tube. Then the upper part of the pressure tube, with the open tip facing downward into the funnel, is filled with distilled water, and this washing is also filtered into the precipitation tube. Finally, the lower part of the pressure tube is filled once more with distilled water, which is filtered and collected as before.

Evaporation of the Sulfate Solution. The filtered sulfate solution collected in the precipitation tube, which, with the washings, will have a volume of 10 to 12 ml., has to be evaporated to dryness. This is conveniently accomplished with the aid of the evaporation apparatus shown in Fig. 44. The precipitation tube containing the sulfate solution is attached to the distillation head of the apparatus and then is immersed in a concentrated salt solution contained in a suitable beaker. After the rest of the apparatus has been assembled, as shown in the illustration, suction is applied; the solution in the beaker is brought to boiling, and after ten to fifteen minutes the contents of the precipitation tube will have been evaporated to dryness.

Transfer of the Sulfate. To the dry residue in the precipitation tube is added 3 ml. of distilled water, and the resulting solution is poured without loss, which is accomplished by slightly greasing the outer rim of the precipitation tube, into the previously weighed porcelain crucible. The precipitation tube is rinsed with three successive portions of 2 ml. of distilled water, and these washings are added to the liquid in the crucible. During this operation the immersion filter remains in the crucible.

Precipitation. The porcelain crucible is placed on a steam bath, the opening of which has been covered with a cloth to protect the crucible from coming in contact with the metal ring of the bath. The crucible is heated on the steam bath for five minutes; then 0.5 ml. of 10% barium chloride solution is added drop by drop; after the crucible has stood on the steam bath for another five minutes it is placed on a clean glass surface, covered with a beaker, and allowed to cool for five minutes.

Filtration. The previously cleaned flat-bottomed test tube * is placed in the suction bottle and the rubber stopper carrying the siphon is inserted. After the stem of the immersion filter is attached to the capillary tip of the siphon sufficient suction is applied so that the solution filters at a rate of 1 or 2 drops per second. After the aqueous solution has been filtered off, the barium sulfate precipitate in the crucible is washed three times with 3 ml. of dilute hydrochloric acid solution (1 : 300) by spraying the wash liquid on the immersion filter and the wall of the crucible, from the rim on downward, while rotating it, so that no part of it is neglected. These washings are also filtered off through the immersion filter as before. Then, without being removed from the crucible, the immersion filter is detached from the capillary end of the siphon and is placed in the crucible, where it remains during the entire process of heating, igniting, and weighing.

---

* It is important that a clean test tube be used, because it is advisable to observe whether particles of the filtrate are passing through the immersion filter; if this is the case, the filtrate collected in the test tube is transferred to the crucible and the filtration is repeated.

Drying and Ignition. After the completion of the filtration the upper part of the stem of the immersion filter and the outside of the crucible are wiped with a clean lint-free cloth, after which it is handled only with the crucible tongs. With the immersion filter remaining in the crucible, it is transferred to a drying oven and heated for five minutes at a temperature of about 120° C. Then it is placed in a larger porcelain crucible provided with a cover, set on a clay triangle, and heated with a gas burner to a dull red.* The heating is continued for five minutes, after which the porcelain crucible is removed from the large crucible, placed on a metal block (the metal block of a microdesiccator will serve this purpose), covered with a beaker, and after five minutes the crucible is transferred to the conical cavity of a microdesiccator. After another five minutes the crucible is placed into a second microdesiccator, left standing near the balance for ten minutes, and then crucible and immersion filter are weighed together to within ±10 micrograms.

Time:	*Minutes*
Weighing of the sample	10
Preparation, filling, and sealing of the pressure tube	15
Digestion	120
(During this time the crucible is dried, ignited, and weighed)	
Transfer and evaporation of the oxidation mixture	20
Precipitation	15
Filtration	10
Drying and igniting	15
Cooling and weighing of the crucible and immersion filter	30
Total	235
Waiting time during digestion	120
Net	115

---

* Instead of a gas burner an electric furnace may be employed, but the temperature of the furnace must be high enough to heat the large crucible, which contains the crucible with the barium sulfate precipitate, to a dull red.

Calculation:

Log of weight of barium sulfate,
Plus log of factor (13782),
Plus negative log of weight of sample;
Antilog of total = percentage of sulfur.

TITRIMETRIC DRY COMBUSTION METHOD

Principle

The organic substance containing sulfur, but not containing halogen or nitrogen, is oxidized in an atmosphere of oxygen in the presence of a platinum catalyst.[12, 17, 44, 46] The sulfur present in the compound is thereby converted into the gaseous oxides of sulfur, which are then absorbed in aqueous hydrogen peroxide solution distributed over a spiral condenser. Thus the final sulfur oxidation product is sulfuric acid, which is titrated with 0.01 *N* sodium hydroxide solution.

(*a*) $\text{Organic S-compound} \xrightarrow[O_2;\ \text{Pt-catal.}]{\Delta} SO_2 + SO_3 + xCO_2 + xH_2O$ (oxidation)

(*b*) $SO_2 + SO_3 + H_2O_2 + H_2O = 2H_2SO_4$ (absorption)

(*c*) $H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O$ (titration)

Apparatus

Oxygen Tank, Gas Wash Bottle, Combustion Spiral Tube, Combustion Stand, Platinum Contacts. These are the same as in the *dry combustion method* for the determination of halogen (p. 160).

Titration Equipment. This is the same as described under " Standard Solutions " (p. 51).

Reagents

Perhydrol (30%).

Hydrogen Peroxide Solution. Twenty milliliters of 30% perhydrol is diluted with distilled water to 100 ml. total volume.

Distilled Water.

0.01 *N Sodium Hydroxide Solution* (p. 56).

Methyl Red Indicator Solution (p. 54).

Procedure

Combustion. The combustion tube is cleaned and dried as described on p. 162. Ten milliliters of hydrogen peroxide solution, previously neutralized with 0.01 *N* sodium hydroxide solution to a canary yellow, is poured into a clean test tube and siphoned about 1 cm. beyond the spiral of the combustion tube. After the spiral has been completely moistened with the absorption liquid by rotating the combustion tube, the excess is permitted to drain off into the test tube and is discarded. Then the combustion tube is placed on the stand, the pre-ignited platinum contacts and the rubber stopper are inserted, and the pieces of wire gauze are slipped over the combustion tube. The part of the tube containing the spiral is protected by placing a precipitation tube over it which is held in position by clamping it to a small stand. Next, the combustion tube is connected to the gas wash bottle and the oxygen tank, and the flow of oxygen is regulated to a delivery of 3 ml. per minute, as described in the corresponding halogen determination on p. 163. Then the long burner is lighted and the platinum contacts are heated to a dull red.

The amount of substance to be taken depends upon the precision of the balance and also upon the percentage of sulfur present in the compound, but enough should be taken to obtain a precipitate weighing 4 to 5 mg. if it is weighed on a microanalytical balance and about twice as much if an ordinary analytical balance is employed. The sample, if a solid, is weighed in the platinum boat; if a liquid, in a capillary pipet which is placed in a platinum foil; it is placed in the combustion tube 6 to 7 cm. from the nearest platinum contact. The combustion is started about 5 cm. from the substance with a slightly hissing flame of a gas burner which is slowly moved towards the sample until the first sign of decomposition of the substance is noticed. The burner is left in this position for ten minutes and then is gradually moved nearer to the sample. The gradual advance of the burner is important, because, if the combustion is carried out too rapidly, uncombusted material may be deposited before the indentation in front of the spiral of the combustion tube. The heating is continued for ten minutes directly under the substance and then the burner is gradually moved forward until it reaches the long burner. The time required for the entire combustion is approximately forty minutes for an average sample and up to one hour for a larger one. After completion of the combustion both burners are extinguished and the combustion tube is allowed to cool in a stream of oxygen. Then the oxygen is turned off, the combustion tube is detached from the gas wash bottle, and the wire gauzes as well as the platinum boat, or foil, and the platinum contacts are removed.

Titration. Eight milliliters of distilled water is placed in the precipitation tube which covered the spiral of the combustion tube, the mouth of which has been provided with a dust filter. This wash liquid is siphoned into the combustion tube to 2 cm. beyond the end of the spiral. The dust filter being closed with a finger, the outlet of the combustion tube is placed in a 125-ml. steamed-out Pyrex glass Erlenmeyer flask, and the wash liquid is allowed to drain off. This procedure of rinsing the combustion tube is repeated twice with the same amount of wash liquid, which is also collected in the Erlenmeyer flask. Then the combustion tube is rinsed once more with about 5 ml. of distilled water, which is sprayed into the orifice of the combustion tube, but this solution is permitted to drain into the precipitation tube, where it is tested for its acidity by adding a trace of methyl red. If, after 1 drop of 0.01 *N* sodium hydroxide solution has been added, the solution turns yellow, indicating that the previous three washings were adequate, as they usually are, then it may be discarded; otherwise it is added to the washings in the Erlenmeyer flask. The solution in the Erlenmeyer flask is boiled for about twenty seconds, another trace of methyl red is added, and then it is titrated with 0.01 *N* sodium hydroxide solution to the end point, which is reached when the solution retains the canary-yellow color for two minutes.

Time:	*Minutes*
Weighing of the sample	10
Preparation and filling of the combustion tube	20
Combustion	40
Cooling of the combustion tube *	15
Transfer and titration of the absorption solution	20
Total	105

Calculation:

Log of ml. 0.01 *N* sodium hydroxide solution,
Plus log of factor (20493),
Plus negative log of weight of sample;
Antilog of total = percentage of sulfur.

* During this time the next sample can be weighed.

Remarks

Wet Combustion Methods. The time-honored wet combustion method for the determination of sulfur in organic compounds as originally devised by L. Carius [10, 26, 38] and as first applied to quantitative micro work by F. Emich and J. Donau [15] has been given preference [1, 36, 40, 56, 57] over both the dry combustion [12, 17, 24, 44, 46] and the fusion methods.[6, 14, 41] The wet combustion method, which may also be used in semi-micro work,[11] is applicable to any and all types of organic sulfur compounds, regardless whether they are very volatile or not volatile at all, and regardless whether in addition to sulfur the substance also contains other elements, such as halogen or nitrogen, or both. The reagents used are easy to purify, and no blank is necessary. The previously often-voiced objection to the Carius method, namely the danger of explosion and the possibility of contaminating the barium sulfate precipitate with glass splinters, have been remedied,[40] the first by reducing the amount of nitric acid used to only a few drops, and the second by filtering the sulfate solution before precipitation. Usually the sulfate is precipitated as *barium sulfate,* but precipitation with benzidine hydrochloride has also proved to be feasible. In the latter case a regular halogen filter tube (p. 152) is used for the filtration, drying and weighing of the *benzidine sulfate* precipitate.[40, 59] The evaporation of the sulfate solution to dryness is best accomplished under reduced pressure and in a closed system;[40] evaporation over free gas flames, or on open steam baths heated by gas, must definitely be avoided.[36] Digestion of non-volatile sulfur compounds in Kjeldahl flasks[4, 29, 32, 48, 49] has been tried, and either potassium hydroxide in combination with hydrogen peroxide[32, 49] or potassium permanganate[4] as well as chlorates and iodates[28, 29] may be used as oxidizing agents.

A. Friedrich[17] has described a gravimetric method for the simultaneous determination of *sulfur* and *halogen,* utilizing the wet combustion method. The organic substance is oxidized in the pressure tube with nitric acid to which a crystal of silver nitrate has been added. The filtrate obtained after filtration of the silver halide precipitate and the washings are collected in a short test tube, rinsed quantitatively into a Pyrex glass dish, and the sulfuric acid is precipitated on a steam bath with 2 to 3 ml. of a 1% solution of barium nitrate. This solution must be tested for halogen with silver nitrate. If it should not be free from halogen, it must be precipitated from the warm solution with silver nitrate and the filtrate added to the solution in the dish. The transfer and filtration of the barium sulfate precipitate are carried out by any of the standard methods. Distilled water is used in the washing procedure instead of the dilute acid solutions.

Dry Combustion Methods. The catalytic dry combustion method for the determination of halogen and sulfur as devised by F. Pregl[44, 46] has been modeled after the semi-micro method of M. Dennstedt.[12] In this method the final oxidation product may be determined *gravimetrically* in all cases, and *titrimetrically* if no interfereing elements such as halogens, or nitrogen, or both, are present. The *gravimetric* determination is carried out as follows:

After the combustion, which is carried out as described on p. 188, the combustion spiral tube is removed from the stand, and its upper part is wiped with a towel. Then it is held in a vertical position, and with the aid of the graduate wash cylinder 3 ml. of dilute hydrochloric acid solution (1 : 300) is sprayed into the orifice of the tube while it is rotated to ensure effective rinsing of its inner surface. To avoid possible loss of absorption liquid during the rinsing, the capillary end of the combustion tube is held over the porcelain crucible. The mouth of the combustion tube is closed with a dust filter, and the tube is held in an inclined position while the absorption liquid is blown into the porcelain crucible; the capillary tip is also rinsed with a few drops of dilute hydrochloric acid solution. The washing of the combustion tube is repeated three times, but with 2 ml. of dilute hydrochloric acid solution, which is also collected in the porcelain crucible. Then it is washed once more with 3 ml. of wash liquid, and this solution is drained into the test tube which served as a cover for the combustion tube during the combustion. A drop of barium chloride solution is added to this wash liquid, and it is observed whether turbidity occurs or not. If an opalescence or precipitate is noted, the liquid is transferred to the porcelain crucible after the original solution therein has been evaporated. The precipitation, filtration, and ignition of the barium sulfate precipitate are carried out as described on p. 186. With proper modifications, such as extraction of the residue left in the boat after combustion, organic metal salts containing sulfur may be analyzed satisfactorily.[2]

A combustion spiral tube in which the *spiral tube* is *detachable* has been described by C. W. Beazley.[7] An arrangement for *continuous operation* without interruption of the heating process has been devised by L. T. Hallett and J. W. Kuipers.[24] The combustion gases are bubbled through the usual hydrogen peroxide solution contained in a suitably constructed absorption tower. At the termination of the combustion process the sulfate solution is rinsed through a stopcock at the bottom of the vertical absorption tower into the appropriate titration or precipitation vessel. The combustion may be carried out with [12, 17, 44, 46] or without the platinum contacts.[23, 33, 47, 51]

According to E. W. D. Huffman [27] the organic substance containing sulfur may also be combusted in an atmosphere of oxygen in a combustion tube containing *metallic silver*. After combustion, the silver sulfate is extracted and the resulting extract is subjected to electrolysis. From the weight of the silver deposited the amount of sulfur originally present in the organic compound may be calculated. Carbon and hydrogen may be determined simultaneously. An *electrolytic oxidation* method for the determination of sulfur in organic compounds has been described by P. Piutti and D. Dinelli.[43]

As with halogens, it is also possible to determine sulfur in organic compounds by destructive *catalytic hydrogenation,* according to H. Ter Muelen and J. Heslinga.[55] The sulfur present is thus converted into hydrogen sulfide, which then may be determined gravimetrically or iodometrically.[20]

Fusion Methods. In the *microbomb method* of A. Elek and D. W. Hill,[14] which is particularly useful for the determination of sulfur in proteins, and which is modeled after the method of S. W. Parr,[41] the organic sulfur compound is fused with a mixture of potassium nitrate, or chlorate, and sodium peroxide, and sugar in a suitably constructed metal bomb (Fig. 42).[2, 6, 14, 41] These oxidizing agents ensure quantitative oxidation of the sulfur to sulfuric acid or alkali sulfates, respectively. The sulfate thus formed is precipitated with barium chloride and the resulting barium sulfate precipitate is determined gravimetrically.

The reagents must be free from sulfur, sulfides, sulfites, or sulfates. The fusion and extraction is carried out as in the corresponding halogen determination described on p. 166. The resulting extract is cooled in an ice bath, and then 5 ml. of concentrated hydrochloric acid is slowly added. The acid solution is filtered, and both test tube and filter are rinsed with distilled water. The filtrate is transferred in 10- to 15-ml. portions to a well-steamed 50-ml. round-bottomed Pyrex glass dish or platinum evaporating dish with a spout; 0.5 ml. of barium chloride solution is added to the liquid in the dish, and the whole is evaporated to dryness. The solid residue is moistened with 10% hydrochloric acid solution, and the evaporation is repeated. The final solid residue is taken up in 30 ml. of distilled water and allowed to stand over night. The procedure of transferring the barium sulfate depends upon whether inverted filtration or filtration by gravity is employed.

Fusion of the organic sulfur compound with sodium peroxide in a *nickel* crucible covered with a weighted lid gives satisfactory results with non-volatile sulfur compounds, but may lead to serious explosions.[34, 45] Also fusion in an *open boat* in presence of a sodium carbonate-cobalt oxide oxidation mixture and in an atmosphere of oxygen has been found to be feasible.[8, 62]

Filtration. For the filtration of the barium sulfate precipitate several methods have been devised.[5, 13, 15, 16, 25, 31, 39, 44, 46, 50, 61] At present the method of *inverted filtration* as originally devised by F. Emich,[15, 16] which subsequently was applied to the filtration of barium sulfate precipitates originating in organic sulfur determinations by W. Saschek,[50] and others,[25, 39] is the simplest and has now become a standard procedure. The barium sulfate precipitate may, however, also be filtered using a regular suction arrangement and a Neubauer crucible.[44, 46] In this case the apparatus (Fig. 47) consists of a 250-ml. suction flask (*a*) fitted with a rubber stopper through which a glass tube (*b*) of 8-cm. length and 1-cm. outside diameter is inserted. The glass tube should have the same outside diameter as the bottom of the Neubauer crucible. A rubber tubing 2 cm. long is placed over the top of the glass tube to form a sleeve (*c*) which holds the crucible (*d*) in place. A rubber tubing approximately 50 cm. long is attached to the side arm of the suction flask; the free end of the rubber tubing is provided with a glass mouthpiece, because the filtration is carried out with suction by mouth.

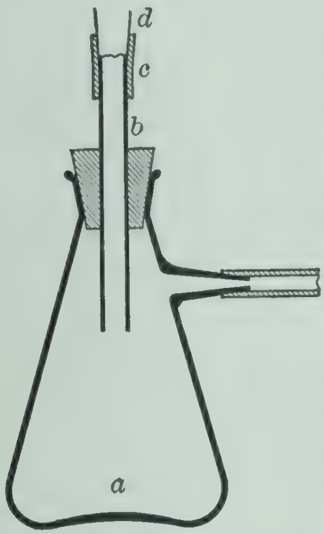


Fig. 47. Filtration Apparatus for Filtration with the Neubauer Crucible. Explanation in Text.

Neubauer Platinum Crucible.[3] The crucible is provided with a lid and a capsule which fits tightly over the bottom. It has a capacity of approximately 3 ml., is 1.4 cm. high, and has a diameter of 1.2 cm. at the rim and 1 cm. at the bottom. The filter mat consists of compressed platinum sponge which, even at a high rate of filtration, completely retains the barium sulfate precipitate. A satisfactory working crucible should permit the passage of an average of 4 ml. of filtrate per minute if suction by mouth is applied.* The inside of the crucible is wiped before every determination with a moist cotton tuft wound around a toothpick (not steel wire) and is repeatedly rinsed with distilled water; it is then placed in the rubber sleeve of the filtration device and washed well with dilute hydrochloric acid. After being removed from the filtration device it is closed with the lid and lower cap, placed on a platinum foil about 3 cm. square, and both are set in a silica triangle. The crucible is first dried on a hot plate or in a drying oven. When dry, the crucible is heated to redness for three minutes with a non-luminous gas flame. The lid is also held in the flame with the platinum-tipped forceps just long enough to heat it to redness. Then the crucible is allowed to stand on a clean metal block for five minutes, after which it is placed on the metal block of the microdesiccator and is weighed fifteen minutes later to ±10 micrograms.

---

* When the filtration becomes too slow, owing to the accumulation of barium sulfate in the pores of the filter mat, the crucible is washed with a small amount of concentrated sulfuric acid. After the crucible has been cleaned in this manner, freshly precipitated barium sulfate is again filtered onto the filter mat which, by means of suction, is rinsed repeatedly with distilled water to ensure that the pores of the filter mat have the proper density.

The time of cooling may be shortened by transferring the crucible to a second metal block several minutes later; the crucible can then be weighed ten minutes after the ignition.

The barium sulfate precipitate may be transferred from the precipitation tube to the Neubauer crucible by means of a dropping pipet provided with a rubber cap[46] or by a medicine dropper.[50] The dropping pipet is about 8 cm. long over all, 1 cm. in diameter, and narrows abruptly to a capillary of 3-cm. length and 1-mm. bore. After the transfer of the precipitate and the wash liquid, the rubber cap is removed and the pipet is alternately rinsed with alcohol and distilled water.

The barium sulfate precipitate may also be transferred to the crucible by means of a snipe feather cemented with Krönig's glass cement into a glass capillary 2.5 mm. in external diameter and approximately 15 cm. long.[44, 46] Particles of the cement which adhere to the outside of the capillary are removed with benzene, and then the feather is washed consecutively with benzene, alcohol, and ammoniacal soap solution. The feather is kept in a stoppered test tube.

A new filtration method has been devised by P. L. Kirk and R. Craig[31] for the filtration of very small amounts of precipitate. O. Wintersteiner[61] has described an automatic filtration apparatus. A new filter crucible and[5] a micro Gooch crucible[13] have also been devised.

Titration. Titration methods may be employed for both the combustion and the fusion methods. Such titration methods[22] may involve benzidine,[17] barium chloride,[32, 49] barium chromate,[4] barium carbonate, barium oxalate, or lead nitrate. Straight *alkalimetric* determinations[18, 21, 32, 44, 46, 49] as well as *iodometric* titrations[4, 20, 21, 28, 37, 58] are in vogue, for the wet,[4, 32, 49, 58] the dry,[18, 21, 44, 46] and also the catalytic hydrogenation methods.[55] *Iodometric* titrations usually involve precipitation of the sulfate with an excess of standard barium chloride solution, followed by precipitation of the excess barium chloride with a standard bichromate solution and final iodometric determination of the excess bichromate as follows:

$$K_2Cr_2O_7 + 6KI + 14HCl = 8KCl + 2CrCl_3 + 7H_2O + 3I_2$$

The use of suitable *absorption indicators*[23, 24, 30, 33, 42, 47, 51–53, 60] such as tetrahydroxyquinone[24] or erythrosine[9] is advocated more and more and has been found particularly useful in industrial work where large numbers of sulfur analyses have to be performed.[24, 33, 47]

Since the *alkalimetric* sulfur determination method as devised by F. Pregl [44, 46] is applicable only to substances free of nitrogen or halogen, attempts have been made to devise titrimetric methods which eliminate this disadvantage. In the method of A. Friedrich and O. Watzlaweck [18] the initial combustion is carried out catalytically; the absorption solution and the washings are collected in a platinum or quartz dish. After the addition of 1 drop of phenolphthalein to the solution 0.02 N sodium hydroxide solution is run in from a buret with constant stirring until a permanent red color is obtained, whereupon the solution is concentrated on the steam bath. After twenty to thirty minutes the concentrated sodium sulfate solution is removed from the steam bath and 0.02 N sulfuric acid solution is run in from a buret, an amount equivalent to the 0.02 N alkali previously taken being used. The solution is stirred well with a glass rod after the addition of the sulfuric acid and again evaporated to dryness on the steam bath. The residue is dissolved in a little water, sprayed in a thin stream from a wash bottle along the edge of the dish, and again evaporated completely to dryness. The crust of salt is dissolved once more in water and the residue is allowed to stand for forty-five minutes on the steam bath. Repeated evaporation (three times) is essential to drive off the volatile acids and to convert the sulfuric acid originally present to sodium bisulfate. The residue is then dissolved in 5 to 8 ml. of hot water, a trace of methyl red indicator solution is added, and the solution is titrated with 0.02 N sodium hydroxide to the first appearance of the yellow color; the dish is then placed on the steam bath for a few minutes and the solution is titrated while still hot to the canary-yellow color.

Owing to the uncertainty whether or not all volatile acids have been completely removed from the bisulfate solution, a blank titration must be made, which, if negative, may be omitted in further analyses of the same or similar substances.

For the blank test, an amount of 0.02 N sulfuric acid equivalent to the total alkali used for the neutralization and for the bisulfate titration is run into the dish containing the sodium sulfate. The contents of the dish are evaporated to dryness on the steam bath; the residue is dissolved in water, allowed to remain on the steam bath for forty-five minutes, and titrated as above with 0.02 N alkali. If all the volatile acids have been removed in the first determination, the sulfuric acid used in the blank test will be titrated back fully; otherwise, a proportionately smaller amount of acid will be found.

Substances containing iodine cannot be determined by this method. An analysis requires three to four hours and is claimed to be accurate to ± 0.2%.

D. J. Gibson and T. H. Caulfield [21] also reported a method by which sulfur and halogen may be determined simultaneously. The combustion products are absorbed in excess standard alkali, and the unused standard solution is titrated back. Mercuric oxycyanide is then added, and the halogen is determined by titration with 0.01 N sulfuric acid solution, methylene blue-methyl orange indicator solution, which permits the observation of the end point more easily than the ferric thiocyanate ordinarily employed, being used. The reaction involved proceeds as follows:

$$\mathrm{Hg(OH)CN + NaCl = NaOH + HgCl(CN)}$$

LITERATURE

1. Aisenstadt, J., *Ind. Org. Chem.* (*Russ.*), **2**, 165 (1937).
2. Alicino, J. F., *Ind. Eng. Chem., Anal. Ed.*, **11**, 298 (1939); **13**, 506 (1941).
3. American Platinum Works, Newark, N. J., U. S. A.
4. Angeletti, A., *Ann. chim. applicata*, **29**, 356 (1939).
5. Bailey, A. J., *Ind. Eng. Chem., Anal. Ed.*, **9**, 490 (1937).
6. Beamish, F. E., *Ind. Eng. Chem., Anal. Ed.*, **5**, 348 (1933).
7. Beazley, C. W., *Ind. Eng. Chem., Anal. Ed.*, **11**, 229 (1939).
8. Brunck, O., *Z. angew. Chem.*, **18**, 1560 (1905).
9. Burg, W. V., *Ind. Eng. Chem., Anal. Ed.*, **11**, 28 (1939).
10. Carius, L., *Ann.*, **116**, 1 (1860); **136**, 129 (1865); **146**, 301 (1868); *Ber.*, **3**, 697 (1870).
11. Clark, E. P., *J. Assoc. Official Agr. Chem.*, **16**, 255 (1933).
12. Dennstedt, M., "Anleitung zur vereinfachten Elementaranalyse," V. Meisner's Verlag, Hamburg, 1919.
13. Dunbar, R. E., *Ind. Eng. Chem., Anal. Ed.*, **9**, 355 (1937).
14. Elek, A., and Hill, D. W., *J. Am. Chem. Soc.*, **55**, 3479 (1933).
15. Emich, F., and Donau, J., *Monatsh.*, **30**, 754 (1909).
16. Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York, N. Y., 1932, pp. 68–75.
17. Friedrich, A., "Die Praxis der quantitativen Mikroanalyse," F. Deuticke, Vienna and Leipzig, 1933, p. 109.
18. Friedrich, A., and Watzlaweck, O., *Z. anal. Chem.*, **89**, 401 (1932).
19. Friedrich, A., and Mandl, F., *Mikrochemie*, **22**, 14 (1937).
20. Gelman, N. E., *Zavodskaya*, **8**, 673 (1939).
21. Gibson, D. J., and Caulfield, T. H., *Mikrochemie*, **17**, 262 (1935).
22. Grant, J., *Chem. Products*, **2**, 125 (1939).
23. Grote, W., and Krekeler, H., *Z. anal. Chem.*, **114**, 321 (1938); **98**, 463 (1934); *Z. angew. Chem.*, **46**, 106 (1933).
24. Hallett, L. T., and Kuipers, J. W., *Ind. Eng. Chem., Anal. Ed.*, **12**, 360 (1940); **11**, 521 (1939).
25. Heller, K., and Meyer, K., *Z. anal. Chem.*, **71**, 117 (1927).
26. Houben, J., "Die Methoden der organischen Chemie," Verlag G. Thieme, Leipzig, 1925, Vol. I, p. 72.
27. Huffman, E. W. D., *Ind. Eng. Chem., Anal. Ed.*, **12**, 53 (1940); *Mikrochemie*, **24**, 218 (1938); **25**, 384 (1938).

28. Josephson, B., *Analyst*, **64**, 181 (1939).
29. Kahane, E., and Kahane, M., *Compt. rend.*, **198**, 372 (1934).
30. Kahler, H. L., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **12**, 266 (1940).
31. Kirk, P. L., and Craig, R., *Ind. Eng. Chem., Anal. Ed.*, **3**, 345 (1931).
32. Kitamura, R., *J. Pharm. Soc. Japan*, **57**, 58, 233 (1937).
33. Koegel, R., *Proc. Pregl Group, Metrop. Microchem. Soc.*, New York, January, 1940.
34. Kraus, H., *Z. anal. Chem.*, **117**, 243 (1939).
35. Kuck, J., and Griffel, M., *Ind. Eng. Chem., Anal. Ed.*, **12**, 125 (1940).
36. Lindenfeld, K., *Mikrochemie*, **16**, 153 (1935).
37. Manov, G. G., and Kirk, P. L., *Ind. Eng. Chem., Anal. Ed.*, **9**, 198 (1937).
38. Meyer, H., "Analyse und Konstitutionsermittlung organischer Verbindungen," J. Springer, Berlin, 1903, p. 161.
39. Miller, Ch. C., *J. Chem. Soc.*, **1939**, 1962.
40. Niederl, J. B., Baum, H., McCoy, J. S., and Kuck, J. A., *Ind. Eng. Chem., Anal. Ed.*, **12**, 428 (1940).
41. Parr, S. W., *J. Am. Chem. Soc.*, **30**, 764 (1903).
42. Peabody, W. A., and Fisher, R. S., *Ind. Eng. Chem., Anal. Ed.*, **10**, 651 (1938).
43. Piutti, P., and Dinelli, D., *Gazz. chim. ital.*, **67**, 133 (1937).
44. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, pp. 131–165.
45. Pringsheim, H., *Ber.*, **36**, 4244 (1903); **38**, 2459 (1905).
46. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son and Co., Philadelphia, Pa., 1937, pp. 116–126.
47. Royer, G. L., *Proc. Pregl Group, Metrop. Microchem. Soc.*, New York, January, 1940.
48. Rudolph, W., *Z. anal. Chem.*, **113**, 325 (1938).
49. Sakamoto, S., *J. Chem. Soc. Japan*, **59**, 631 (1939).
50. Saschek, W., *Ind. Eng. Chem., Anal. Ed.*, **9**, 491 (1937).
51. Schöberl, A., and co-workers, *Z. angew. Chem.*, **50**, 334 (1937).
52. Schroeder, W. C., *Ind. Eng. Chem., Anal. Ed.*, **5**, 403 (1933).
53. Sheen, R. T., and Kahler, H. L., *Ind. Eng. Chem., Anal. Ed.*, **8**, 27 (1936); **10**, 206 (1938).
54. Staatliche Porzellanmanufaktur, Berlin, Form No. 0.9886.
55. Ter Meulen, H., and Heslinga, J., "Neue Methoden der organisch-chemischen Analyse," Akad. Verlagsgesellschaft, Leipzig, 1927.
56. Tiedcke, C., *Mikrochemie*, **23**, 301 (1938).
57. Unterzaucher, J., *Mikrochemie*, **18**, 312 (1936).
58. Werner, A., *Z. angew. Chem.*, **52**, 139 (1939).
59. Weygand, C., and Hennig, H., *Chem. Fabrik*, **9**, 8 (1936).
60. Wilson, C. W., and Kemper, W. A., *Ind. Eng. Chem., Anal. Ed.*, **10**, 418 (1938)
61. Wintersteiner, O., *Mikrochemie*, **2**, 14 (1924).
62. Young, G. H., *Ind. Eng. Chem., Anal. Ed.*, **10**, 686 (1938).

VIII. DETERMINATION OF PHOSPHORUS

Principle

The organic substance is decomposed either by fusion with suitable solid oxidation mixtures or by digestion with a mixture of perhydrol and concentrated sulfuric acid. The phosphorus present in the organic substance is converted to phosphoric acid, which is determined gravimetrically as *ether-dried* ammonium phosphomolybdate.[12]

(*a*) Organic P-compound $\xrightarrow{\text{oxid.}}$ $H_3PO_4 + xCO_2 + xH_2O$ (oxidation)

(*b*) $H_3PO_4 \xrightarrow[\text{HNO}_3]{(\text{NH}_4)_2\text{MoO}_4} (NH_4)_3PO_4 \cdot 14MoO_3$ (precipitation)[13b]

Apparatus

Precipitation Tubes. Pyrex glass test tubes approximately 100 ml. in total capacity, about 4.5 cm. in diameter, and 20 cm. high, and with markings at 15-ml. and at 30-ml. levels, are used.

Filter tubes. These are the same as described in the Carius method for the determination of halogen (p. 152). If a sintered-glass filter tube is used, it should be tested for its retentivity of the ammonium phosphomolybdate precipitate.

Filtration Apparatus. This is the same as described in the Carius method for the determination of halogen (p. 153).

Wash Bottles. One wash bottle each for alcohol, ether, acetone, ammonium nitrate solution, and distilled water is required.

Vacuum Desiccator.

Kjeldahl Digestion Flasks. These are the same as described under "Volumetric Determination of Aminoid Nitrogen" (p. 71).

Digestion Oven. This is the same as described under "Volumetric Determination of Aminoid Nitrogen" (p. 70).

Silver Crucible. This crucible is 1.8 cm. in lower and 3.3 cm. in upper diameter and is 4 cm. high.

Bomb. This is the same as is used for the corresponding halogen determination (p. 165).

Reagents

Ammonium Nitrate. C.P.

Ammonium Sulfate. C.P.

Ammonium Molybdate. C.P.

Sodium Peroxide. C.P.

Potassium Hydroxide. C.P.

Potassium Nitrate. C.P.

Sucrose, C.P., or *d-Glucose,* C.P.

Concentrated Nitric Acid (sp. gr.: 1.36).

Dilute Nitric Acid (1 : 1).

Concentrated Sulfuric Acid (sp. gr.: 1.84).

Perhydrol (30%).

Ethyl Alcohol (95%).

Diethyl Ether (anhydrous). The ether should be free of alcohol, and 1 ml. of water should dissolve in 150 ml. of ether at room temperature to give a clear solution.

Acetone. Free of aldehydes.

Distilled Water.

Molybdate Solution. Fifty grams of ammonium sulfate is placed in a 1-liter volumetric flask and dissolved in 500 ml. of concentrated nitric acid (sp. gr.: 1.36); 150 g. of powdered ammonium molybdate is dissolved in 400 ml. of boiling distilled water, cooled to room temperature, and poured slowly, with constant stirring, into the ammonium sulfate-nitric acid solution. The flask is then filled up to the mark with distilled water, allowed to stand for three days, and filtered through an ordinary filter into a glass-stoppered brown reagent bottle; it is kept in a dark, cool place.

Nitric-Sulfuric Acid Mixture. Thirty milliliters of concentrated sulfuric acid (sp. gr.: 1.84) is poured into 1 liter of nitric acid (sp. gr.: 1.19–1.21), which is obtained by mixing 420 ml. of nitric acid (sp. gr.: 1.40) with 580 ml. of distilled water.

Aqueous Solution of Ammonium Nitrate (2%). The solution should be slightly acidic; 1 or 2 drops of nitric acid may be added if necessary.

Fusion Mixtures:

(*a*) Potassium hydroxide and potassium nitrate (5 : 1).
(*b*) Potassium nitrate and sucrose (3 : 1).

Procedure

Wet Combustion.[10] The substance is weighed in the weighing tube and transferred to a clean and dry Kjeldahl digestion flask; 0.5 ml. of concentrated sulfuric acid and 0.5 ml. of perhydrol are added to the sample and the mixture is heated over the flame of a microburner, or in series determinations, on the digestion oven until fumes of sulfur trioxide begin to appear. This procedure is repeated at least twice with the addition of a few drops of perhydrol each time. Concentrated nitric acid may be substituted for the perhydrol if the substance appears not to be oxidizable with the concentrated sulfuric acid-perhydrol mixture alone (fats, etc.). When the digestion mixture is clear, it is allowed to cool and then it is diluted with 1 to 2 ml. of distilled water. The resulting solution is rinsed into the precipitation tube, 1 to 2 ml. of distilled water being used for each rinsing. Nitric-sulfuric acid mixture is added to the solution in the precipitation tube to bring the total volume to 15 ml.

(*a*) *In the Silver Crucible.*[2] The substance is weighed in the weighing tube and transferred to the crucible. Approximately 1.2 g. of potassium nitrate-potassium hydroxide mixture is added to the sample and the mixture is fused by heating the upper part of the crucible with a small non-luminous flame while the crucible is rotated and then slowly lowered into the flame. In about three minutes the fusion is complete, as shown by the clearness of the melt. The crucible is covered and allowed to cool by standing on a metal block for four or five minutes. Slight pressure on the walls of the crucible will loosen the melt, which is then transferred to the precipitation tube. The crucible is washed three or four times with about 2 ml. of distilled water, and the washings are also collected in the precipitation tube. Enough of the nitric-sulfuric acid mixture is added to the solution to bring the volume up to 15 ml.

(*b*) *In the Bomb.*[3] The substance is fused in the bomb as described in the respective bomb method for the determination of halogen (p. 165). After the bomb is cool, the melt is dissolved in as small a volume of distilled water as possible. During the cooling, and with caution, 5 ml. of the nitric-sulfuric acid mixture is slowly added. The mixture is then quantitatively transferred to the precipitation tube; it must be filtered if cloudy or turbid. Enough of the nitric-sulfuric acid mixture is added to bring the total volume of the solution to 15 ml.

Precipitation.[9, 13a, 14] The precipitation tube containing the reaction mixture, as obtained in any of the three oxidation methods given above, is heated on the steam bath for ten minutes. Then 15 ml. of the freshly filtered molybdate reagent is run into it from a pipet without touching the wall of the precipitation tube. After two to three minutes the mixture is shaken thoroughly and allowed to stand for at least six hours. Very small amounts of phosphorus require a considerably longer time, e.g., 0.5 mg. of phosphorus up to eighteen hours, and less than 0.5 mg. up to thirty-six hours.

Filtration. The filter tube is prepared before being weighed by placing it on the filtration apparatus and washing it with water, hot dilute nitric acid, and distilled water; the last traces of distilled water are removed by rinsing the filter tube twice with alcohol and twice with acetone and finally with ether. The filter tube is wiped with a moist flannel and dry chamois and placed in a desiccator, which is evacuated on the water pump; no drying agents are used in the desiccator. The filter tube is removed from the desiccator thirty minutes later and weighed five minutes afterwards. The ammonium phosphomolybdate precipitate is transferred to the filter tube by means of the same filtration arrangement as is used for the filtration of the silver halide (p. 152). After the supernatant liquid has been siphoned off, the precipitate is washed thoroughly with 2% ammonium nitrate solution and transferred to the filter tube. To remove the last traces of precipitate from the wall of the precipitation tube, it is rinsed alternately with the 2% ammonium nitrate solution and 95% ethyl alcohol. The filter tube is washed twice with alcohol, then with acetone, and finally with ether. After wiping, the filter tube is placed in the desiccator, which is evacuated. It is left in the desiccator for thirty minutes, and five minutes after its removal it is weighed to ±20 micrograms.

Time:

	Minutes
Weighing of the sample	10
Digestion or fusion	30
Precipitation *	15
Washing and drying of the filter tube	40
Weighing of the filter tube	10
Filtration	15
Drying of the filter tube	30
Weighing of the precipitate	10
Total	160

Calculation:

Log of weight of precipitate,
Plus log of factor (16209),
Plus negative log of weight of sample;
Antilog of total = percentage phosphorus.

* The waiting time necessary for complete precipitation is not included in this time schedule.

Remarks

In addition to the *wet combustion* method for the determination of phosphorus in organic compounds, as devised by H. Lieb and O. Wintersteiner,[10] and the two *fusion* methods, as devised by A. Elek [2] and A. Elek and D. W. Hill,[3] other fusion methods are known [1, 9, 13a, 14] which have been used with varying success.

The phosphorus in organic compounds may also be determined *volumetrically*.[5, 6, 8] The organic substance is decomposed either by fusion or wet combustion, and the precipitation is carried out in a 100-ml. beaker. After standing over night, the supernatant liquid is filtered through a hard filter; then the precipitate is washed with 4 to 5 ml. ice-cold 50% alcohol and rinsed from the filter back into the beaker. The precipitate is dissolved in 0.1 N alkali, twice as much as will just dissolve it being taken. The ammonia is driven off by boiling the solution for at least thirty minutes. Two or three drops of 1% phenolphthalein indicator solution and an excess of about 4 ml. of 0.1 N acid are added to the alkaline solution, after which the solution is boiled for approximately fifteen seconds, then cooled and titrated back with 0.1 N sodium hydroxide solution to the usual end point.

The reaction involved in the titration of the ammonium phosphomolybdate is as follows: [7]

$$(NH_4)_3PO_4 12MoO_3 \cdot 2HNO_3 + 28NaOH =$$

$$12Na_2MoO_4 + 2NaNO_3 + Na_2HPO_4 + 16H_2O + 3NH_3$$

The phosphoric acid, as produced in the wet combustion or in fusion procedures, may also be *precipitated* as *strychnine phosphomolybdate.*[4] The ratio of phosphorus to strychnine phosphomolybdate is 1 : 89. Since the precipitation is quantitative even in the cold, it can be filtered after thirty minutes. If the organic substance also contains arsenic acid it is removed before precipitation by distilling off the arsenic trichloride in a slow stream of hydrogen chloride. According to whether the phosphorus is determined gravimetrically or nephelometrically, the oxidation mixture is evaporated down with nitric acid or hydrochloric acid, respectively.[8]

A *Drop-Scale* procedure for the determination of phosphorus has been described by R. Lindner and P. L. Kirk.[11] The method is based on the acidimetric titration of the ammonium phosphomolybdate. The quantities of phosphorus determined ranged from 0.5 to 9 micrograms.

Other methods for the determination of phosphorus in organic compounds involve *colorimetric* methods,[17] the *measuring* of the ammonium phosphomolybdate *precipitate* (±5%),[16] and determination as *magnesium pyrophosphate.*[15]

LITERATURE

1. Aisenstadt, J., *Zavodskaya Lab.*, **6**, 1014 (1937).
2. Elek, A., *J. Am. Chem. Soc.*, **50**, 1213 (1928).
3. Elek, A., and Hill, D. W., *J. Am. Chem. Soc.*, **55**, 3479 (1933).
4. Embden, G., *Z. physiol. Chem.*, **113**, 138 (1920).
5. Feigl, F., Strebinger, R., and Barrenscheen, H. K., *Mikrochemie*, **7**, 116–142 (1929).
6. Friedrich, A., "Die Praxis der quantitativen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, p. 119.
7. Iverson, P., *Biochem. Z.*, **104**, 23 (1920).
8. Kuhn, R., *Z. physiol. Chem.*, **129**, 64 (1923).
9. Lieb, H., *see* Pregl, F., and Fyleman, E., "Quantitative Organic Microanalysis," Second English Edition, P. Blakiston's Son & Co., Philadelphia, Pa., 1930, p. 151.
10. Lieb, H., and Wintersteiner, O., *Mikrochemie*, **2**, 78 (1924).
11. Lindner, R., and Kirk, P. L., *Mikrochemie*, **22**, 300 (1937).
12. Lorenz, v., N., *Landwirtschaftl. Versuchstat.*, **55**, 183 (1901); *Z. anal. Chem.*, **46**, 193 (1907); **51**, 161 (1912).
13. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, (*a*) pp. 167–173; (*b*) p. 170.
14. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., pp. 126–131.
15. Schuhecker, K., *Z. anal. Chem.*, **116**, 14 (1939).
16. Vila, A., *Compt. rend.*, **198**, 657 (1934).
17. Zambotti, V., *Mikrochemie*, **26**, 113 (1939).

IX. OTHER ELEMENTS

Existing micromethods for the quantitative determination of metals and non-metals in both inorganic and organic compounds have been extensively reviewed by A. A. Benedetti–Pichler (metals, 130 papers; [4a] non-metals,[157] papers [4b]) and by C. J. van Nieuwenburg.[22]

In the following an account is given of micromethods for the quantitative determination of elements which are of lesser importance in quantitative organic elementary analysis than those presented in the preceding chapters.

Arsenic

The organic substance containing arsenic is oxidized similarly to compounds containing phosphorus. Either *wet combustion* [15, 16, 20, 23a, 25a, 38] (Carius method, see p. 151), or *Kjeldahl digestion* method, p. 200, or *fusion* [3] (bomb method, p. 201) is employed. The arsenic acid formed is determined either (*a*) *gravimetrically* as magnesium pyroarsenate, or (*b*) *iodometrically* [14, 35] as follows:

(*a*) $AsO_4^{---} + Mg^{++} + NH_4^+ \rightarrow MgNH_4AsO_4$

$$2MgNH_4AsO_4 \xrightarrow{\Delta} H_2O + 2NH_3 + Mg_2As_2O_7$$

(*b*) $H_3AsO_4 + 2HI = H_2O + H_3AsO_3 + I_2$

The following reagents are necessary: concentrated sulfuric acid (sp. gr.: 1.82), concentrated nitric acid (sp. gr.: 1.42), concentrated hydrochloric acid (sp. gr.: 1.19), perhydrol, magnesia reagent (5.5 grams magnesium chloride and 10.5 grams ammonium chloride dissolved in 100 ml. of distilled water), ammonium hydroxide solution (2 *N*), sodium peroxide, potassium iodide.

Precipitation and Filtration. The oxidation mixture, as obtained in the wet combustion or fusion method, is rinsed into a Pyrex glass evaporating dish and then is evaporated to dryness on a steam bath. The residue is dissolved in 3 to 4 ml. of 2 *N* ammonium hydroxide solution, and 1 ml. of magnesia reagent is added. The precipitate is amorphous at first, and to permit it to become crystalline it is left standing for at least six hours. The magnesium ammonium arsenate is transferred to a Neubauer platinum crucible with the aid of a feather and is filtered as described in the corresponding determination of sulfur (p. 194). The last traces of precipitate are transferred from the glass dish to the crucible by rinsing the dish alternately with 2 *N* ammonium hydroxide solution and ethyl alcohol. After all liquid has been filtered off, the precipitate in the crucible is washed with 3 ml. of 2 *N* ammonium hydroxide solution. Then the crucible is removed from the filtration apparatus, closed with the lid and lower cap, and strongly ignited on a platinum cover. The magnesium pyroarsenate still contains traces of magnesium salts at this point and, therefore, must be washed again with dilute ammonium hydroxide solution. After the crucible has been re-ignited it is placed on the metal block of the microdesiccator and is weighed after ten minutes. An accuracy of ±0.2% is claimed for this method.

Titration. The oxidation mixture, as obtained in the Kjeldahl digestion process or by the bomb method, is rinsed with distilled water into a ground-glass-stoppered 125-ml. Erlenmeyer flask and heated to boiling for a few seconds. Two milliliters of 4% potassium iodide solution is added to the arsenic solution in the Erlenmeyer flask which is stoppered and the mixture is allowed to stand for ten minutes. The liberated iodine is then titrated with 0.01 *N* sodium thiosulfate solution. When the solution is faintly yellow, the liquid is diluted to about 20 ml. with freshly distilled water, 4 or 5 drops of starch indicator solution are added, and the titration is completed. A faint reddish tint is considered to be the end point. It is usually necessary to correct for a blank, but such a correction should not exceed 0.3 ml. of the 0.01 *N* sodium thiosulfate solution.

Calculation:

Gravimetric:

Log of weight of precipitate,
Plus log of factor (68368),
Plus negative log of weight of sample;
Antilog of total = percentage arsenic.

Iodometric:

Log of ml. 0.01 *N* sodium thiosulfate solution,
Plus log of factor (57380),
Plus negative log of weight of sample;
Antilog of total = percentage arsenic.

Boron in organic compounds may be determined *titrimetrically* as boric acid[24] after fusion of the organic boron compound with sodium carbonate in a platinum crucible. Organic compounds containing **copper**[18, 23b, 25b] or **mercury**[10, 19, 34] are subjected to wet combustion and the resulting oxidation mixture is subjected to *electrolysis*, by using plain platinum electrodes for copper and gold-plated platinum electrodes for mercury, in the electrolysis apparatus of F. Pregl,[23b, 25b] which recently has been improved by A. Llacer, J. A. Sozzi, and A. A. Benedetti–Pichler.[18] **Mercury** may also be determined gravimetrically [21] after the organic mercury compound has been subjected to dry combustion and then the liberated mercury absorbed in a suitably constructed absorption tube containing gold leaf.

Oxygen

Of the several methods for the direct determination of oxygen in organic compounds the semi-micro method of H. Ter Meulen and J. Heslinga,[30] which has been repeatedly modified for micro work, appears thus far to have found the widest application.[1, 8, 9, 11a, 13, 17, 26, 32, 33] The method involves destructive hydrogenation of the organic substance with gravimetric or titrimetric [13, 17] determination of the water formed. The reactions involved are as follows:

$$CO + 3H_2 = CH_4 + H_2O$$

$$CO_2 + 4H_2 = CH_4 + 2H_2O$$

Thus, for each oxygen atom present in the compound, one mole of water is formed.

Of the micromethods, the procedure devised by J. Unterzaucher and K. Bürger,[33] which follows rather closely the apparative arrangement and procedure of the regular carbon and hydrogen method (p. 101), appears to be the simplest. The apparatus consists of a hydrogen tank, followed by a drying tube filled with phosphorus pentoxide, a Friedrich-type pressure regulator (p. 134), a preheater containing a copper spiral; another drying tube follows, which in turn is attached to the hydrogenation tube—a regular side-arm combustion tube. The hydrogenation tube contains silver wool, the *cracking* agent (platinized powdered quartz), calcium oxide, a nickel spiral, and the *hydrogenation catalyst*, which consists of a mixture of activated nickel (prepared from nickel formate), thorium oxide, and powdered quartz. A Blumer-type vertical absorption tube (p. 137), with ground-glass joints and filled with phosphorus pentoxide, is used for the absorption of water. The customary Mariotte flask terminates the apparative arrangement. The combustion and sweeping-out process requires 100 ml. of hydrogen at a speed of 10 ml. per minute. The total time for one determination, including the wiping and weighing of the absorption tube, is about fifty minutes.

Other methods involve gasometric determination of the residual oxygen after complete dry combustion of the substance,[11] pyrolysis of the substance in an atmosphere of nitrogen,[27,40] and various wet combustion methods [5,7,28,29,36,37] involving "organic oxidation equivalent analysis" [5,36,37] and the determination of "oxygen values." [28]

Selenium in organic compounds may be determined *gravimetrically.* A suitable micro procedure, based upon the earlier methods of F. Wrede [39] and of H. D. K. Drew and C. R. Porter,[6] has been devised by H. K. Alber and J. Harand [2] and S. Umezawa.[31] The organic compound is combusted catalytically in an atmosphere of oxygen in a spiral combustion tube as in the respective catalytic combustion method for halogen (p. 160). The selenious acid thus formed is reduced to elementary selenium by means of sulfur dioxide in hydrochloric acid solution, and the precipitated selenium is filtered with the usual halogen filter tube (p. 152), which may be provided with a suitable cap.

Very small amounts of **silver** may be determined *photometrically* [12] as silver dichromate.

LITERATURE

1. Afanasev, B. N., *Zavodskaya Lab.*, **6**, 551 (1937).
2. Alber, H. K., and Harand, J., *J. Franklin Inst.*, **228**, 243 (1939).
3. Beamish, F. E., and Collins, H. L., *Ind. Eng. Chem., Anal. Ed.*, **6**, 379 (1934).
4. Benedetti-Pichler, A. A., "Die Fortschritte der Mikrochemie in den Jahren 1915 bis 1926," G. Klein and R. Strebinger, Vienna, 1927; (*a*) pp. 311–341; (*b*) pp. 341–372.
5. Christensen, B. E., and Facer, J. F., *J. Am. Chem. Soc.*, **61**, 3001 (1939).
6. Drew, H. D. K., and Porter, C. R., *J. Chem. Soc.*, **1929**, 2091.
7. Glockler, G., and Roberts, L. D., *J. Am. Chem. Soc.*, **50**, 828 (1928).
8. Goodloe, P., and Frazer, J. C., *Ind. Eng. Chem., Anal. Ed.*, **9**, 223 (1937).
9. Ivanei, I. F., *J. Applied Chem.* (*U.S.S.R.*), **12**, 470 (1939).
10. Jacquemain, R., and Devillers, G., *Bull. soc. chim.*, (5) **5**, 1338 (1938).
11. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **6**, 358 (1934); **7**, 363 (1935); **8**, 57 (1936); (*a*) **9**, 535 (1937).
12. Krainick, H. G., *Mikrochemie*, **26**, 158 (1939).
13. Lacourt, A., *Bull. soc. chim. Belg.*, **46**, 428 (1937); *Compt. rend.*, **205**, 280 (1937).
14. Leipert, T., *Mikrochemie, Pregl Festschrift*, 1929, p. 266.
15. Lieb, H., "Abderhaldens Handbuch der biochemischen Arbeitsmethoden," Urban and Schwarzenberg, Berlin and Vienna, 1919, p. 727.
16. Lieb, H., and Wintersteiner, O., *Mikrochemie*, **2**, 80 (1924).
17. Lindner, J., *Ber.*, **59**, 2561, 2806 (1926); **70**, 1025 (1937); **71**, 1382 (1938).
18. Llacer, A., Sozzi, J. A., and Benedetti-Pichler, A. A., Detroit Meeting, Am. Chem. Soc., September, 1940; *Ind. Eng. Chem., Anal. Ed.*, **13**, 507 (1941).
19. Lomholt, S., and Christiansen, J. A., *Biochem. Z.*, **81**, 356 (1917).
20. Mayr, C., and Kerschbaum, E., *Z. anal. Chem.*, **73**, 321 (1928).
21. Meixner, A., and Kröcker, F., *Mikrochemie*, **5**, 131 (1927).
22. Nieuwenburg, C. J. van, *Chem. Weekblad*, **35**, 799 (1938).

23. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, (*a*) p. 175; (*b*) p. 185.
24. Roth, H., *Z. angew. Chem.*, **50**, 593 (1937).
25. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., (*a*) p. 132; (*b*) p. 141.
26. Russel, W. W., and Marks, M. E., *Ind. Eng. Chem., Anal. Ed.*, **8**, 453 (1936).
27. Schuetze, M., *Z. anal. Chem.*, **118**, 245 (1939).
28. Stanek, V., and Nemes, T., *Z. anal. Chem.*, **95**, 244 (1933).
29. Strebinger, R., *Z. anal. Chem.*, **58**, 97 (1919).
30. Ter Meulen, H., and Heslinga, J., "Neue Methoden der organisch-chemischen Analyse," Akad. Verlagsgesellschaft, Leipzig, 1927.
31. Umezawa, S., *Bull. Chem. Soc. Japan*, **14**, 153 (1939).
32. Unterzaucher, J., *Ber.*, **73**, 39 (1940).
33. Unterzaucher, J., and Bürger, K., *Ber.*, **70**, 1382, 1392 (1937); **71**, 429 (1938); *Chem. Fabrik*, **1940**, 305.
34. Verdino, A., *Mikrochemie*, **6**, 5 (1928).
35. Vieböck, F., and Brecher, C., *Ber.*, **63**, 3207 (1930); **65**, 493 (1932).
36. Williams, R. J., *J. Am. Chem. Soc.*, **59**, 288 (1937).
37. Williams, R. J., and co-workers, *J. Am. Chem. Soc.*, **59**, 291 (1937).
38. Wintersteiner, O., *Mikrochemie*, **4**, 155 (1926).
39. Wrede, F., *Z. physiol. Chem.*, **109**, 272 (1920).
40. Zimmermann, W., *Z. anal. Chem.*, **118**, 258 (1939).

DETERMINATION OF MOLECULAR WEIGHT

I. EBULLIOSCOPIC METHODS

Principle

The elevation of the boiling point of a known, pure organic solvent, caused by a known amount of a solute (the sample) with which it must not react, is determined by means of a suitably constructed Beckmann thermometer. The various apparatus are designed so that the determination may be carried out with small amounts of material—1.5 to 5 ml. of solvent and from 10 to 25 mg. of solute. Proper apparative arrangement is provided to ensure maximum constancy of temperature.

Apparatus

Pregl's Apparatus (Fig. 48, *a*, *b*).[3, 6a] This consists of a stand to hold the microburner (*a*) and a round metal plate (*b*) 9 cm. in diameter, provided with concentric rings for the support of three concentric glass cylinders (c_1, c_2, and c_3) which surround the boiling chamber (*d*) of the apparatus. The dimensions of the cylinders, which serve to provide an evenly heated and well-circulated air current, thereby ensuring maximum constancy of temperature, are as follows: c_1, the outer cylinder, is 14 cm. high and 8.4 cm. in diameter; the middle cylinder, c_2 is 12 cm. high and 4.8 cm. in diameter; the innermost cylinder, c_3, has a constriction in the middle, is 11 cm. high, 2.6 cm. in diameter at the top, and 3.6 cm. in diameter at the base. The chamber which extends into the neck of cylinder c_3 is 6 cm. high, 1.8 cm. in diameter, and contains the solvent, the solute, and the platinum tetrahedrons. Cylinder c_4, which serves the purpose of diverting the air current rising from the microburner, is 2.6 cm. high and 3.6 cm. in diameter and is made of mica or any heat-resistant transparent material. Its top has an opening just wide enough to permit the insertion of the boiling chamber. The cylinder is held in place by three narrow strips of asbestos paper which are inserted between it and cylinder c_3. The vessel carries two long side arms, one 10 cm. in length, with a ground-glass cap for the insertion of the sample, and a second one 13 cm. in length, serving as a reflux condenser. A Beckmann thermometer, with a range of 3°, divided into 0.01°, is inserted into the neck of the boiling chamber by means of a tightly fitting cork. The apparatus is supported by two horizontal clamps fastened to the adjustable vertical column of the stand and has a suitably perforated celluloid shield for protection against drafts from above. Platinum tetrahedrons are introduced into the chamber (*d*) to prevent superheating.

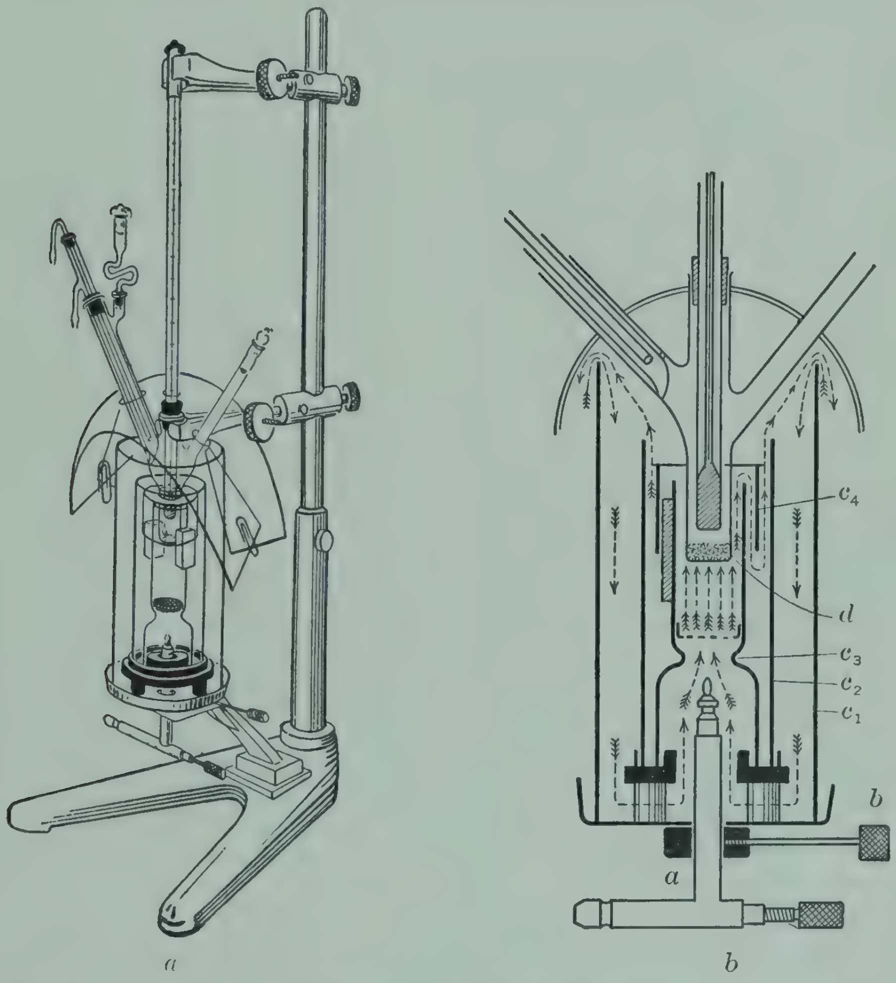


FIG. 48. *a*, Pregl Molecular-Weight Apparatus, Assembled; *b*, Diagram. Explanation in Text.

Rieche's Apparatus (Fig. 49, *a* and *b*).[5] This apparatus has only one side arm (*a*), which serves as a reflux condenser as well as for the introduction of the sample through a vertical opening (*b*). To prevent superheating and to ensure a maximum constancy of temperature, so vital in this determination, the apparative arrangement is such that the micro

Beckmann thermometer, which is identical with the one used in the Pregl apparatus, is surrounded by the boiling solution and also by the vapor of the solvent. Newer modifications ensure this to a still higher degree.[5a] A side arm (*c*) is attached to the boiling chamber (*d*) to provide for the necessary circulation of the boiling liquid. Platinum tetrahedrons (0.3 gram) are introduced into the bulb to minimize superheating of the reaction mixture.

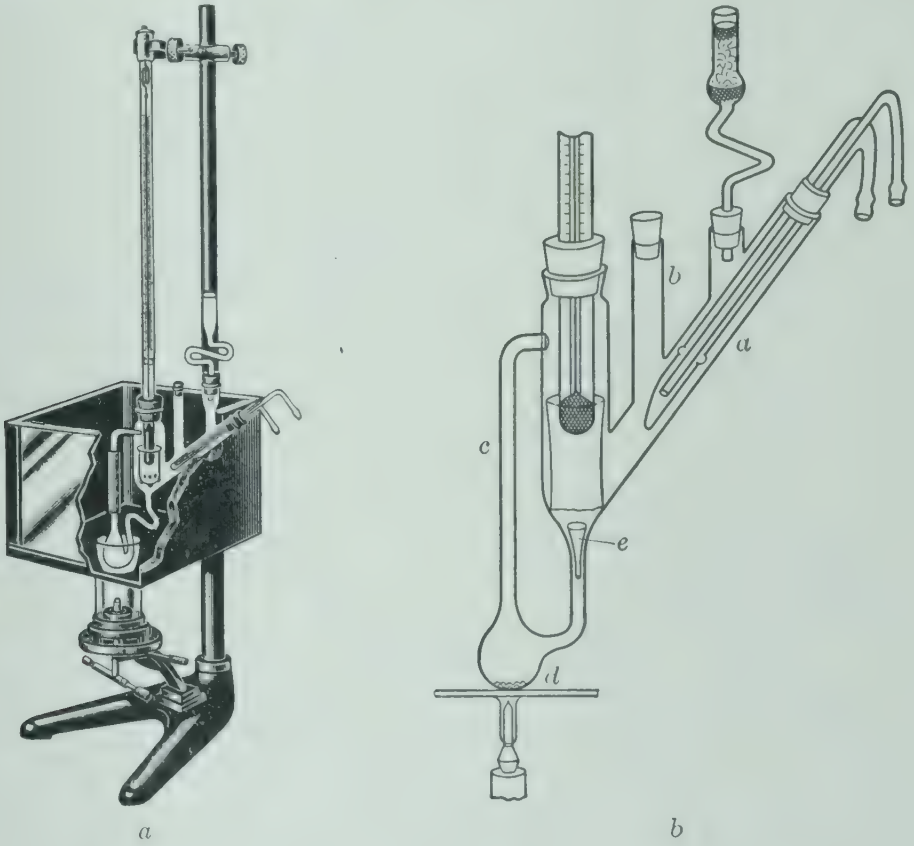


Fig. 49. *a*, Rieche Molecular-Weight Apparatus; *b*, Diagram. Explanation in Text.

Sucharda–Bobranski–Schmitt Apparatus (Fig. 50).[7, 9] The assembled two-piece apparatus has an overall length of 12.5 cm. and is 25.5 cm. high. It consists of: (*a*) the pear-shaped boiling chamber, which is 3.5 cm. high and 1.7 cm. in widest diameter, with fused-in glass splinters to ensure even boiling; (*b*) an undulating tube of 9-cm. length and 5-mm. diameter; two thermometer cups (*c* and *d*) of which the inner (*c*) is 2 cm. in diameter and 4 cm. in height and the outer (*d*) is 2.5 cm. in diameter and 6.5 cm. in height which is provided with a receptacle (*e*) 4.5 cm. deep for the insertion of the micro Beckmann thermometer;

(*f*) the reflux condenser, the base of which is connected with the thermometer cups by a straight siphon tube (*g*) 8.5 cm. long and 8 mm. in diameter. The condenser is also connected with the boiling chamber by means of a suitably constructed overflow tube (*h*), which is 3 mm. in diameter. The condenser is 18 to 20 cm. long, has an inside diameter of 1 cm., and is provided with a water jacket and a standard ground-glass joint.

Precision Pipets (1.5, 4.0 and 5.0 ml.).

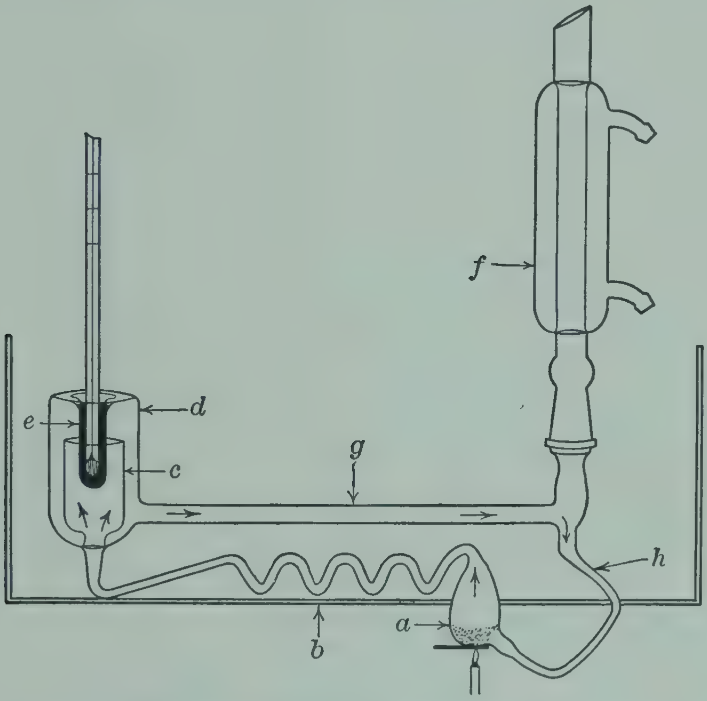


FIG. 50. Sucharda-Bobranski-Schmitt Apparatus. Explanation in Text.

Solvents

Only solvents of highest purity can be used. The usual solvents for these determinations are: acetone, absolute ethyl alcohol, ether, benzene, glacial acetic acid, chloroform, or water.

Procedure

PREPARATION OF THE SAMPLE

Solids. It is advisable to use the sample in the form of a pellet, which is prepared with the aid of the pellet press. About 10 mg. is used for the Pregl apparatus and 15 to 25 mg. for a determination with the Rieche or Sucharda–Bobranski–Schmitt apparatus. The sample, pressed into a single pellet, is weighed in a weighing tube and need not be weighed more accurately than to ±0.01 mg.

Liquids. Weighing pipets or capillaries as used in the vaporimetric molecular weight determination (p. 46) are employed.[6b]

PREPARATION OF THE APPARATUS

Pregl [3, 6a] *and Rieche* [5] *Apparatus.* The platinum tetrahedrons are first introduced into the boiling chamber, the Beckmann thermometer is inserted, and the reflux condenser is attached. The solvent, 1.5 ml. for the Pregl apparatus and 4 ml. for the Rieche apparatus, is measured accurately with a precision pipet and is introduced next. The apparatus is slowly heated by means of a microburner; careful regulation of the flame is absolutely essential for the success of the determination. The Beckmann thermometer, previously adjusted to the boiling-point temperature of the solvent, is read to 0.002° with the aid of a magnifying lens, and as soon as two consecutive readings, taken at an interval of two to three minutes, are identical (T_1), the sample is added through the side arm. No changes in the procedure of heating are made during this operation and the subsequent observations. Ten minutes later the Beckmann thermometer is again read, and the end point is reached when two consecutive readings, taken at a time interval of several minutes, are the same (T_2). If necessary, the sample may be recovered after the determination by transferring the reaction mixture to an evaporating dish and evaporating the solvent.

Sucharda-Bobranski-Schmitt Apparatus.[7, 9] About 5 ml. of solvent and 18 mg. of solute are necessary for one determination. Approximately 3 to 4 ml. of mercury is required to surround the mercury bulb of the Beckmann thermometer, which is inserted in the outer cup and held by a clamp. Under the boiling chamber there should be an asbestos wire gauze and a microburner provided with a chimney. An asbestos board is placed under the apparatus, and when low-boiling solvents, such as acetone, are used, it is necessary to surround the entire apparatus with asbestos board.

These precautions are necessary in order to be able to regulate the heating of the boiling liquid to constant temperature and also to avoid superheating of the solvent. The flame of the microburner is regulated so that the liquid gently overflows the rim of the inner cup. Occasionally it may be necessary to heat the outside of the cup with a small flame of a gas burner, especially when a large amount of solvent accumulates at the bottom of the cup and thereby prevents free circulation of the liquid. This may occur at the beginning of the experiment. Five to seven minutes after regular circulation of the boiling solution has been established, the temperature readings should not vary, more than ± 0.002° C. When this constancy of temperature has been reached, then the boiling point of the solvent is recorded; the microburner is removed for five minutes, and the sample is added to the solvent. If the sample is added in the form of pellets, it may be added through the condenser; however, if the sample consists of small crystals or is in form of a powder, then the condenser is removed and the sample is added directly to the solvent. Some substances cannot be pressed into pellets and hence the ground-glass joint at the base of the condenser is of advantage.

After the introduction of the substance the microburner is again placed under the boiling chamber; the sample is dissolved in the solvent, which is heated continuously until the boiling liquid circulates freely through the apparatus. Three to five minutes later the elevation of the boiling point of the solvent is read to within ±0.002° C., and when constancy of temperature has been obtained, as ascertained by a second reading several minutes later, the determination is completed.

It is very important that the entire apparatus be shielded from drafts or currents of air; unless this precaution is observed accurate results cannot be obtained, which is particularly true of low-boiling solvents; with benzene and such solvents having a higher boiling point there is less danger of inaccuracy.

Calculation:

Log of molecular boiling-point elevation constant (see p. 299).
Plus log of weight of sample,
Plus negative log of weight of solvent,
Plus negative log of observed elevation of boiling point;
Antilog of total = molecular weight.

Remarks

For all three methods presented in this manual, both good [3, 6a, 7, 9] and unsatisfactory results have been claimed.[1, 8] At present the Sucharda-Bobranski ebullioscopic method,[9] which was improved by R. B. Schmitt,[7] seems to be more widely used than either the Pregl [3, 6a] or the Rieche method.[1, 5]

Aside from modifications of the Pregl ebullioscopic method,[4] a new micro ebullioscopic molecular-weight determination method, based upon the corresponding macromethod of A. W. C. Menzies and S. L. Wright,[2] has been developed by J. H. C. Smith and H. W. Milner.[8]

In these methods great difficulty is experienced in maintaining an *absolutely* constant temperature. Most of the time the temperature continues to rise slowly and constantly. It is therefore recommended that not only the boiling points but also the time of observation be recorded. Then, in determining the rise in the boiling point (Δ), only the boiling points recorded after the same time interval should be compared, i.e., the boiling point of the solvent obtained after ten minutes is compared with the boiling point of the solvent and solute after ten minutes, etc. Another way to overcome this general difficulty is to use two apparatus, one with the pure solvent and one with the solution, and observe the respective boiling points at ten, fifteen, twenty, and thirty minutes. The increments at equal elapses of time are then averaged for the final calculation.

The molecular weight of an unknown or a new compound should be determined with at least two different solvents and, if possible, should be checked by either the cryoscopic, the vaporimetric, or the isothermic method.

LITERATURE

1. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933, p. 182.
2. Menzies, A. W. C., and Wright, S. L., *J. Am. Chem. Soc.*, **43**, 2309, 2314 (1921).
3. Pregl, F., "Quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, p. 225.
4. Rezek, A., *Mikrochemie*, **18**, 109 (1935).
5. Rieche, A., *Ber.*, **59**, 218 (1926); *Chem. Ztg.*, **52**, 923 (1928); (*a*) *Mikrochemie*, **12**, 129 (1933).
6. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, (*a*) p. 229; (*b*) p. 242.
7. Schmitt, R. B., *Bull. Am. Assoc. Jesuit Scientists*, **17**, 76 (1939).
8. Smith, J. H. C., and Milner, H. W., *Mikrochemie*, **9**, 117 (1931).
9. Sucharda, E., and Bobranski, B., "Halbmikromethoden zur automatischen Verbrennung organischer Substanzen und ebullioskopische Molekulargewichtsbestimmung," Braunschweig, 1929, p. 135.

II. CRYOSCOPIC METHOD

Principle

The substance to be analyzed is dissolved in a suitable solid organic solvent, and the resulting depression of the melting point of the solvent is observed.[9–11] Essential requirements are ease of crystallization of the solvent and solubility of the solute in the solvent; furthermore, the solute must not react with the solvent, nor should it decompose at the temperature of the melting point of the solvent. By using solvents with a very high molecular melting-point depression constant,[5] such as camphor or borneol,[6, 8] the technic involved is reduced to elementary simplicity and involves merely the taking of a melting point in the conventional manner with the necessary precautions against superheating and changes in the concentration of the reaction mixture.

Apparatus

An ordinary melting-point apparatus, with the exception of the Thiele apparatus, can be used, although a Fisher melting-point apparatus [3] is preferable. An Anschütz thermometer with the proper temperature range (100° to 180° for camphor, or 150° to 220° for borneol) and subdivided into 0.2°, is most suitable. Concentrated sulfuric acid, dibutylphthalate, or Crisco is employed as the bath liquid.

Solvents

Camphor is most commonly used as a solvent. It should be of the highest purity, and it is advisable to resublime it. It is kept in a well-stoppered wide-mouth reagent bottle. Its melting point, as well as the molecular melting-point depression constant, should be redetermined for every fresh batch of camphor by using a known and pure solute, such as azobenzene or naphthalene. These constants may vary, depending on whether the camphor employed is natural or made synthetically, and wide variations have been reported in the literature. The same holds true for borneol,[8] which may also be used in this type of molecular-weight determination.

Procedure

Solids. A thin-walled conical capillary, of the dimensions given in Fig. 51, is sealed in such a way that a thick bottom is avoided. This capillary (*a*) is wiped with a chamois and weighed after several minutes. It is most conveniently handled with the chamois-tipped capillary forceps, or with the chamois finger cots. Then about 1 mg. of substance is introduced and the capillary is weighed again. The substance is introduced as follows: The sample is placed on a small watch glass and taken up with another capillary (*b*), which fits into (*a*) and has a fire-polished orifice. Capillary (*b*) is wiped clean on the outside and then it is placed sufficiently far down into (*a*) to effect a clean introduction when the substance is pushed out with a thin, fire-polished glass rod. It is obvious that serious errors would result if some of the substance were scattered all along the side wall of capillary (*a*), where it could not be mixed with the solvent. Next, an amount of solvent, which, depending upon the molecular weight of the substance, should be 10 to 20 times the amount of sample used, is introduced in the same manner, but with another and somewhat wider capillary. Capillary (*a*) is then weighed again, the weight of the solvent being thus obtained.

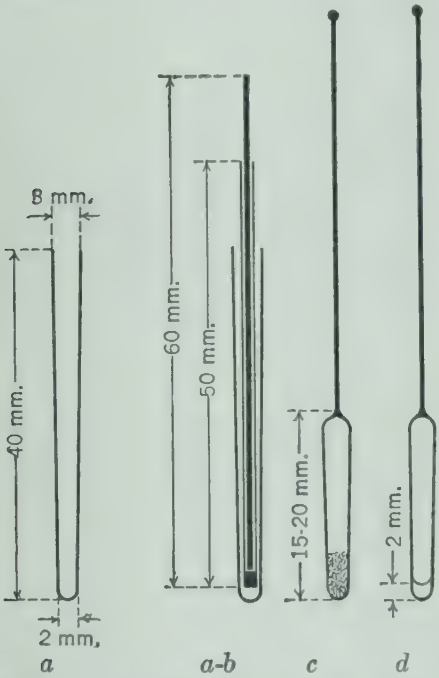


FIG. 51. *a*, Conical Capillary; *a-b*, Capillary *b* Inserted into *a* for the Introduction of the Solvent or Substance; *c*, Capillary *a* Sealed; *d*, Solvent and Substance Molten in Capillary *a*.

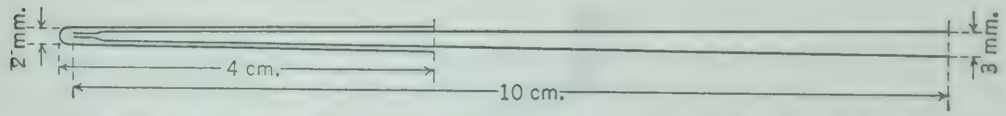


FIG. 52. Pipet for the Introduction of Liquids into the Conical Capillary.

Liquids.[9, 11, 12] Liquids are introduced into the conical capillary with the aid of a suitable pipet shown in Fig. 52, with adequate precaution against losses of the liquid solute due to evaporation, adhesion to the walls of capillary (*a*), etc. In many cases it is advisable to introduce the solid solvent first and to add the liquid solute after the weight of the solid solvent has been determined.

Heating. After the weight of capillary (a), together with that of the solute and the solvent, has been determined, it is sealed as follows: The capillary is held into the non-luminous flame of a microburner 1 to 1.5 cm. above the camphor and heated, while being rotated, until the wall of the capillary collapses to a solid glass rod, which after it is removed from the flame, is drawn out as shown in Fig. 51c. The capillary is then attached to a thermometer calibrated to 0.02°, so that the bottom of the capillary is flush with the end of the thermometer and both are immersed deeply enough in the heating bath of the melting-point apparatus so that the sealed end of the capillary is about 1 cm. below the level of the bath liquid. The bath liquid is heated by means of a microburner to the melting point of camphor, then allowed to cool to the freezing point of the solute-solvent mixture, after which the heating is repeated; this is usually sufficient to mix the solvent thoroughly with the sample. The mixture is again permitted to cool and then it is heated very slowly, at a rate of 0.5° per minute, and the melting point is noted when the last crystal disappears, which happens shortly after the lattice of the crystals collapses and sinks to the bottom of the capillary. The cooling and melting are repeated until a check in temperature of ±0.2° is obtained. The melting point of the solvent is taken at the same time and under the same conditions by attaching a similar capillary containing only camphor to the thermometer; in this manner stem corrections, as well as possible errors due to inaccuracies of the thermometer, are avoided. With some substances the freezing point of the solute-solvent mixture is more readily observable than the melting point, owing to a better visibility of the onset of crystallization, in which case, of course, the freezing point of camphor is also determined under identical conditions. The calculation, whether the freezing point of camphor or the depression of the freezing point is used, remains the same.

Calculation:[6]

Log of molecular melting-point depression constant (see p. 299),
Plus log of weight of sample,
Plus negative log of weight of solvent,
Plus negative log of observed depression of melting point or freezing point;
Antilog of total = molecular weight.

Remarks

It is often advisable to observe the melting point as well as the freezing point of the mixture and the pure solvent; then T_1 and T_2

must be either melting points or congealing points, respectively. Of the many modifications suggested,[1, 2, 4, 7, 8, 13] the methods employing sealed capillaries have been found superior, because concentration changes due to sublimation of the solvent are largely eliminated.

LITERATURE

1. Böhme, H., and Schneider, E., *Z. angew. Chem.*, **52**, 58 (1939).
2. Fang, H. Y., and Shah, P. T., *J. Chinese Chem. Soc.*, **4**, 429 (1936).
3. Fisher, H. L., "Laboratory Manual of Organic Chemistry," Third Edition, John Wiley & Sons, New York, 1934, p. 23.
4. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, pp. 182–186.
5. Jouriaux, M., *Bull. soc. chim.*, **11**, 722, 993 (1912); *Compt. rend.*, **154**, 1692 (1912).
6. Küster-Thiel, "Logarithmische Rechentafeln für Chemiker," W. de Gruyter & Co., Berlin and Leipzig, 1935, p. 83.
7. Liu, Y. P., and Chou, T. P., *J. Chinese Chem. Soc.*, **4**, 422 (1936).
8. Pirsch, J., *Ber.*, **65**, 862, 1227, 1839 (1932); **66**, 349, 506, 815, 1694 (1933); **67**, 101, 1115, 1303 (1934); **68**, 67 (1935); *Z. angew. Chem.*, **51**, 73 (1938).
9. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, p. 237.
10. Rast, K., *Ber.*, **55**, 1051, 3727 (1922).
11. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 237–244.
12. Soltys, A., *ibid.*, pp. 241–242.
13. Tiedcke, C., *Mikrochemie*, **18**, 223 (1935).

III. VAPORIMETRIC METHOD

Principle

The principle involved in this method is the vaporization of the organic substance in a closed system in such a manner that its vapors displace an equal volume of mercury under controlled conditions.[21] The displaced mercury is determined gravimetrically. The necessary corrections for pressure, temperature, and expansion of the mercury and apparatus being applied, the volume of the vapors under standard conditions is determined and from this volume the vapor density or molecular weight of the substance is calculated. Two types of apparatus are used, one for low-boiling liquids and another for higher-boiling liquids or for solid substances.

Apparatus

For Low-Boiling Substances (Fig. 53). This apparatus is employed for liquids boiling below 100° C., and consists of a flask (*a*) of about 100-ml. capacity. A tube (*b*), 10 cm. long and of 5- to 7-mm. bore, sealed to the upper part of the flask, serves as the receptacle for the thermometer (*c*). A side arm (*f*) of 3-mm. bore is connected to the bottom of the flask by a ground-glass joint (*e*); it is long enough to extend 1 to 2 cm. above the surface of the bath and then is bent horizontally for a length of about 6 cm. The arrangement of the flask in the heating bath (*g*) and the position of the receiver for the mercury (*h*) are shown in the illustration.

For High-Boiling Substances (Fig. 54). This apparatus is used for liquids or solids boiling up to 330° C.; it consists of a round-bottomed 350-ml. Pyrex flask (*a*), which serves as the container for the bath liquid. The neck is sealed tangent to the surface of the flask and is 7 to 8 cm. long, with an inner diameter of about 2 cm. By means of an interchangeable ground-glass joint (No. 15) either a Liebig water condenser or a plain air condenser (*b*) of 50-cm. length and with a large enough inner diameter (10 to 12 mm.) to permit the insertion of a thermometer (*c*) is connected to the flask. The Pyrex glass vaporizer (*d*), a slightly elongated or egg-shaped bulb of 12- to 15-ml. capacity, has a stem (*e*) 11 cm. long and 6 mm. in inner diameter sealed to its bottom at an angle

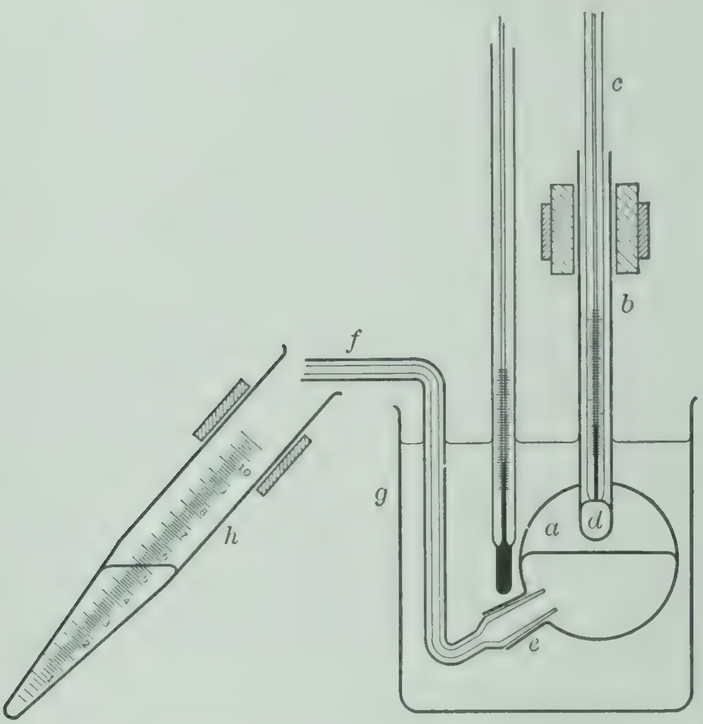


FIG. 53. Vaporimetric Molecular-Weight Apparatus for Low-Boiling Substances. Explanation in Text.

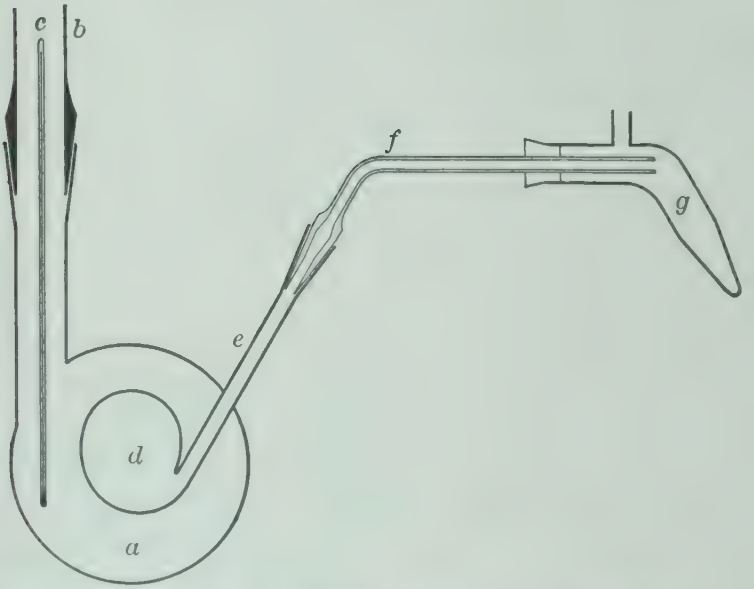


FIG. 54. Vaporimetric Molecular-Weight Apparatus for High-Boiling Substances. Explanation in Text.

of 45 degrees. To facilitate the introduction of the capillary containing the sample, the inner diameter of the bend between the vaporizer and the stem must be about 1 mm. wider than the inner diameter of the rest of the stem. The capillary outlet tube (*f*) is bent at an angle of 135 degrees and has an outer diameter of 5 mm. and an inner diameter of 2 mm. Its horizontal arm is about 10 cm. long; its oblique arm together with the interchangeable ground-glass joint is 5 cm. long. Between this ground-glass joint and the bend are two glass hooks for the attachment of steel springs leading to similar glass hooks on the stem to prevent the loosening of this connection during the determination. The entire vaporizer is fused to the heating flask opposite its neck at an angle of about 45 degrees in such a manner that the glass bulb is in the center of the heating flask and the orifice of the ground-glass joint extends at least 5 cm. beyond the heating flask. The thermometer is introduced through the condenser into the bath liquid and is held in place by a flexible wire. A centrifuge tube of about 15-ml. capacity, which is bent downward at an angle of about 135 degrees below the side arm, serves as the receiver (*g*) for the displaced mercury. By means of a suitably bent wire this receiver is attached to the top hook of the weighing pan of the analytical balance when it is weighed.

Mercury. The mercury used as the sealing liquid must be free from volatile substances. Of the many purification procedures the following appears to be entirely adequate. The mercury (commercial, C.P., or reclaimed) is first treated with concentrated sulfuric acid and then permitted to fall from a funnel, the stem of which is drawn out to a fine opening, in a fine stream into a 250-ml. graduate cylinder filled with dilute nitric acid (1 : 10). After this treatment it is placed in a strong separatory funnel and washed repeatedly with water, acetone, and finally with distilled water. Then the water is removed and the mercury placed in a thick-walled, round-bottom flask which is fitted with a capillary as in a distillation under reduced pressure. To remove the last traces of moisture the flask is then attached to a water suction pump and heated for 1 hour in an oil or sand bath at 250° C. Redistillation of the purified mercury may be substituted for the above procedure of heating.

Procedure

LOW-TEMPERATURE APPARATUS (Fig. 53)

Preparation of the Sample. The sample, which invariably is a liquid, is introduced by means of a capillary weighing pipet, as in the carbon and hydrogen determination, the introduction of potassium chlorate,[24, 27] however, being omitted.

Heating. Since this apparatus is most practicable for liquids boiling below 100° C., only liquid samples, introduced and weighed in capillary pipets as described above, need to be considered. The stem of such a capillary pipet is cut off just below the center bulb and the pipet is centrifuged to ensure against liquid remaining in the hair-fine ending. Then the pipet is cut about 1 mm. above the center bulb (see Fig. 55*a*), and the bulb containing the substance together with the hair-fine ending is placed in the vaporizer, which afterwards is filled with mercury up to the orifice of the ground-glass joint. The side arm is then attached and the entire capillary of the side arm is completely filled with mercury, as described for the high-temperature apparatus. The apparatus, with the receiver attached so that the side arm extends 2 to 3 cm. into the receiver, is placed in a beaker of water and allowed to reach equilibrium in temperature, that is, the thermometer in the water bath and the one in the top tube, which contains some mercury, have to register identical temperatures (T_1). Then the water in the beaker is heated with a gas burner to boiling, the heating being continued until the two thermometers again show the same temperature (T_2). The apparatus is cleaned and dried in the same manner as the high-temperature apparatus.

HIGH-TEMPERATURE APPARATUS (Fig. 54)

Preparation of the Sample

Solids. Capillaries (*b*, *c*) of not less than 1.5-mm. inner diameter are prepared by drawing out a soft-glass test tube in a Méker burner. These capillaries are cut to a length of about 8 cm., wiped with a chamois, placed on the hooks of the left-hand weighing pan of the balance, and weighed after several minutes. Such capillaries weigh about 150 mg. and are counterpoised either by another capillary or by the standard weights. The solid substance is melted on a microscope slide, on a watch glass, or in a capillary of wider bore. The weighed capillary (*b*) is brought in contact with the molten substance, which is allowed to rise in the capillary to a height of 3 to 4 mm.; this is equivalent to 5 to 8 mg. of substance. The capillary is then withdrawn and allowed to cool; if crystallization does not take place upon cooling, it is brought in contact with the remaining solid sample. After crystallization has set in, the outside of the capillary is wiped clean, placed on the hooks of the weighing pan of the balance, and weighed again. If the substance is a solid which does not melt, but sublimes, a melting-point capillary (*c*) of 2-mm. bore and sealed at one end is used. The weighed capillary, open at one or both ends, is cut about 2 mm. above the substance

(Fig. 55*b*, *c*), and the filled capillary, which should be short enough to pass the bend of the vaporizer, is introduced.

Liquids. Capillaries (*b*) open at both ends are used for liquids of high viscosity or syrups or semi-solids. Otherwise capillary pipets (*a*) are used. The stem of the capillary is cut off below the center bulb and the pipet is centrifuged to remove all liquid from its hair-fine end. Then it is cut above the center bulb as shown in Fig. 55*a*, and the bulb containing the substance is placed in the vaporizer. Special precautions are necessary when analyzing very volatile liquids; ice water is put in the flask previous to the introduction of the sample and the flask is stoppered. The mercury and the sealed capillary are cooled in a similar manner.

Filling the Apparatus. Clean and dry mercury is poured into the vaporizer already containing the sample, while the entire apparatus is held over a suitable trough. The filling is accomplished with a minimum of spilling by using a Kells-Ringer flask or similar device having double outlets, one of which has a fine opening. By proper tilting of the flask, the entire bulb and the stem up to the ground-glass joint are easily filled with mercury. The joint of the capillary outlet tube is greased sparingly, and the tube is inserted into the stem of the vaporizer, the outlet tube being filled about one-third with mercury. The steel springs are then attached and the outlet tube is filled completely with mercury by means of a pipet, which is prepared by drawing out a soft-glass tubing to a fine enough capillary so that it fits about 2 cm. into the capillary outlet tube. This pipet is provided with a rubber cap, and by proper inflation a small amount of mercury is drawn into the pipet. The pipet is inserted into the capillary outlet tube and a small amount of mercury is introduced by applying pressure to the rubber cap. The column of mercury introduced in this manner is joined to the mercury column in the capillary outlet tube by inserting and withdrawing a fine copper or platinum wire. Finally, the weighed receiver is attached to the capillary outlet as shown in Fig. 54, and the entire apparatus is clamped to a suitable stand which is also provided with a ring clamp and wire gauze. Sufficient bath liquid to cover about two-thirds of the bulb of the vaporizer is introduced next; small pieces of porous tile are added to ensure even boiling, and the condenser is attached. A thermometer is

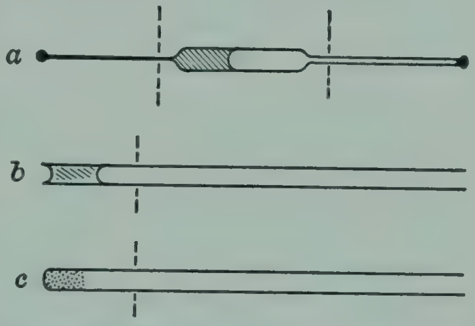


Fig. 55. Pipet and Capillaries for the Vaporimetric Molecular-Weight Determination. Explanation in Text.

inserted through the condenser into the bath liquid so that its mercury bulb is completely immersed. The apparatus is allowed to reach equilibrium of temperature, and then the starting temperature, T_1, is noted. Any mercury which may have entered the receiver during this period is removed and the receiver re-attached.

Heating. After the starting temperature, T_1, has been read, the apparatus is heated rather rapidly to near the boiling point of the bath liquid. The heating is carried out by applying a free flame, or using a suitable heating bath of oil, sand, or Wood's metal. As soon as the bath liquid begins to boil, the heating is reduced to a minimum and the boiling continued for exactly two minutes, at which time the thermometer inside the condenser is again read to give the end temperature T_2. Then the heating is discontinued and the apparatus permitted to cool. The receiver containing the displaced mercury is removed and weighed on a macroanalytical balance to ±0.1 gram.

Either apparatus can be easily refilled, if repetition of the determination is desired, by dipping the delivery tube into mercury before cooling. As the vapors in the vaporizer condense, the mercury will be drawn into the flask. This procedure permits a check determination without the introduction of a new sample. Furthermore, it is possible to determine both the boiling and the condensation point of the substance, namely, upon heating and upon cooling.

Cleaning the Apparatus. The bath liquid in the flask is poured into a suitable container and the flask stoppered. The capillary outlet tube is detached while the apparatus is held over a trough to collect any spilled mercury. The mercury from the vaporizer is emptied into a suitable bottle and at the same time the capillary from a previous determination is removed. About 2 ml. of acetone is introduced into the vaporizer, and the apparatus is well shaken, while its opening is stoppered with a suitable cork. The solvent is removed, and this operation is repeated three times. The apparatus is then placed in an oven heated to about 100° C., and, while it is still warm, the outlet of the vaporizer is attached to a suction pump and evacuated.

Correction for Heat Expansion. By performing the experiment as described above with an empty capillary while the apparatus is heated from T_1 to T_2, the amount of mercury expelled as the result of the expansion of the mercury and glass and of other factors, $c(T_2 - T_1)$, is determined. From the amount of mercury displaced at several temperature points—three are sufficient—a graph may be plotted and the amount of mercury collected from this particular apparatus ascertained for any temperature up to the boiling point of mercury.

Calculation:

Log of 62351 (79385),
Plus log of weight of sample,
Plus log of 373.2 (57194),
Plus negative log of pressure of vapor (see p. 300),[16]
Plus negative log of volume of vapor (see p. 300);
Antilog of total = molecular weight.

Remarks

The low-temperature apparatus (Fig. 53) has proved entirely satisfactory for temperatures up to 100° C.,[21a, b] and so far no substance with a boiling point below this temperature has been encountered which did not give satisfactory results. Even very reactive substances such as alkyl chlorides, bromides, iodides, sulfides and acids, where possible interaction of the mercury with their vapors might be anticipated, gave normal values. At higher temperatures (200° C. and above) difficulties with the ground-glass joint at the bottom of the apparatus were experienced, and hence the form of the apparatus was changed by placing the ground-glass joint above the heating bath.[21d, e] Further improvements were a fused-in thermometer with three graduations (100, 200, and 300° C.), but difficulties in obtaining constancy of temperature finally led to the construction of the high-boiling apparatus shown in Fig. 54.[21c]

Although the vapors of the substance are not in contact with air, a fact which lessens the danger of possible oxidation, they are under higher (about 100 mm.) than atmospheric pressure. This pressure may be diminished by means of partial evacuation by attaching the side arm of the receiver to a suction pump with provision for the maintenance of a constant, although lower, pressure (about 300 mm.). The filling of the vaporizer may also be carried out by evacuation of the vaporizer containing the non-volatile sample and by suitable arrangement allowing the mercury to enter this portion of the apparatus. The boiling point as well as condensation point, which can be observed simultaneously while carrying out the determination, are comparable with the results obtained with other microboiling-point methods already known;[1, 5, 9, 11, 14, 25–29, 31] corrections for the higher pressure (p_1 and p_2) are necessary, however. By slightly enlarging the bulb of the vaporizer without changing the apparatus otherwise, R. B. Schmitt[30] has shown that larger samples (10 to 20 mg.) can be used, which need only to be weighed to ±0.05 mg., a precision obtainable with any ordinary analytical balance, thereby eliminating the use of a microanalytical balance. W. Saschek and F. Schneider[28] have modified the apparatus so that the pressure of the vapors at constant volume is determined.

In the literature a number of microvaporimetric methods have been reported. Most of them are exact miniature editions of the corresponding macromethods. Thus the classical V. Meyer method [19] served as a model for the corresponding micromethod of W. Nernst,[20] and later A. C. Bratton and H. L. Lochte [7] demonstrated the applicability of this method to milligram samples of organic substances. Other micromethods involve the basic principle of the method of A. W. Hofmann [13] and the many modifications of both,[2–4, 6, 8, 12, 15, 17, 18, 20, 22, 24] while E. W. Blank [4] described a micro procedure based upon the classical Dumas method.[10, 23]

LITERATURE

1. Benedetti-Pichler, A. A., and Spikes, W. F., "Introduction to the Microtechnique of Inorganic Qualitative Analysis," Microchemical Service, Douglaston, N. Y., 1935.
2. Biltz, H., *Ber.*, **30**, 1208 (1897).
3. Blackman, Ph., *J. Phys. Chem.*, **11**, 681 (1907); **13**, 532, 606 (1909); **15**, 869 (1912); **24**, 225 (1926); *Ber.*, **41**, 768, 881, 1588, 2487, 4141 (1908).
4. Blank, E. W., *Mikrochemie*, **13**, 149 (1933).
5. Bleier, F., and Kohn, L., *Monatsh.*, **20**, 505 (1899).
6. Booth, H. S., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **2**, 182, 227 (1930); **4**, 427 (1932).
7. Bratton, A. C., and Lochte, H. L., *Ind. Eng. Chem., Anal. Ed.*, **4**, 365 (1932).
8. Chapin, T., *J. Phys. Chem.*, **22**, 337 (1918).
9. DeCeuster, P., *Natuurwe. Tijdschr.*, **15**, 189 (1934).
10. Dumas, A., *Ann. chim.*, (2) **33**, 342 (1826).
11. Emich, F., *Monatsh.*, **38**, 219 (1917). Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York, 1932.
12. Henderson, W. E., *J. Am. Chem. Soc.*, **34**, 553 (1912).
13. Hofmann, A. W., *Ber.*, **1**, 198 (1868); **9**, 1304 (1876); *Z. anal. Chem.*, **8**, 83 (1869); **16**, 479 (1877).
14. Jones, H. C., *J. Chem. Soc.*, **33**, 175 (1878).
15. LaCoste, W., *Ber.*, **18**, 2122 (1885).
16. Landolt-Börnstein, "Physikalisch-chemische Tabellen," J. Springer, Berlin, 1923, Vol. I, p. 76; Vol. II, p. 1335.
17. Lumsden, T. S., *J. Chem. Soc.*, **83**, 342 (1903).
18. Lunge, G., and Neuberg, O., *Ber.*, **24**, 729 (1891).
19. Meyer, V., *Ber.*, **9**, 1216 (1876); **10**, 2068 (1877); **11**, 1867, 2254 (1878); *Z. anal. Chem.*, **16**, 482 (1877); **17**, 373 (1878); **18**, 294 (1879).
20. Nernst, W., *Göttinger Nachrichten*, **1903**, pp. 75–82.
21. (*a*) Niederl, J. B., *Z. anal. Chem.*, **77**, 169 (1929); (*b*) Niederl, J. B., *Ind. Eng. Chem., Anal. Ed.*, **7**, 214 (1935); (*c*) Niederl, J. B., Trautz, O., and Plentl, A., *ibid.*, **8**, 252 (1936); (*d*) Niederl, J. B., and Saschek, W., *Mikrochemie*, **11**, 237 (1932); (*e*) Niederl, J. B., and Routh, I. B., *ibid.*, **11**, 251 (1932).

22. Peak, D. A., and Robinson, R. A., *J. Phys. Chem.*, **38**, 941 (1934).
23. Petterson, O., and Eckstrand, G., *Ber.*, **13**, 1191 (1880).
24. Porter, C. W., *J. Am. Chem. Soc.*, **34**, 1290 (1912).
25. Pregl, F., and Fyleman, E., "Quantitative Organic Microanalysis," P. Blakiston's Son & Co., Philadelphia, Pa., 1930, p. 70. Pregl, F., and Roth, H., "Die quantitative organische Mikroanalyse," J. Springer, Berlin, 1935, p. 63.
26. Richter, R., *Pharm. Ztg.*, **56**, 436 (1915).
27. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, p. 50.
28. Saschek, W., and Schneider, F., Rochester Meeting, Am. Chem. Soc., September, 1937.
29. Schleiermacher, A., *Ber.*, **24**, 944 (1891).
30. Schmitt, R. B., *Bull. Am. Assoc. Jesuit Scientists*, Loyola College, Baltimore, Md., **14**, 69 (1936).
31. Siwoloboff, A., *Ber.*, **19**, 795 (1886).

IV. ISOTHERMIC METHOD

Principle

This method of determining molecular weights is based upon isothermic distillation of volatile solvent from a solution of lower molarity to one of higher molarity. In a closed system this produces measurable changes in the volumes of such solutions. These volume changes are determined by following under a low-power microscope the changes in the position of the meniscuses of the two solutions contained in separate capillaries. By appropriately pairing standard solutions of various but known molarities with a solution of known concentration of a test substance in the same solvent and tabulating the ensuing changes in the volumes of the standard solutions, the molarity of the solution of the test substance is ascertained.

Apparatus

Microscope. An ordinary low-power microscope is used. (Objective 4.6x, ocular 7x.) The eyepiece carries a micrometer scale marked from 0 to 50. These 50 divisions correspond to 1.5 mm.; therefore 1 division corresponds to 0.03 mm.

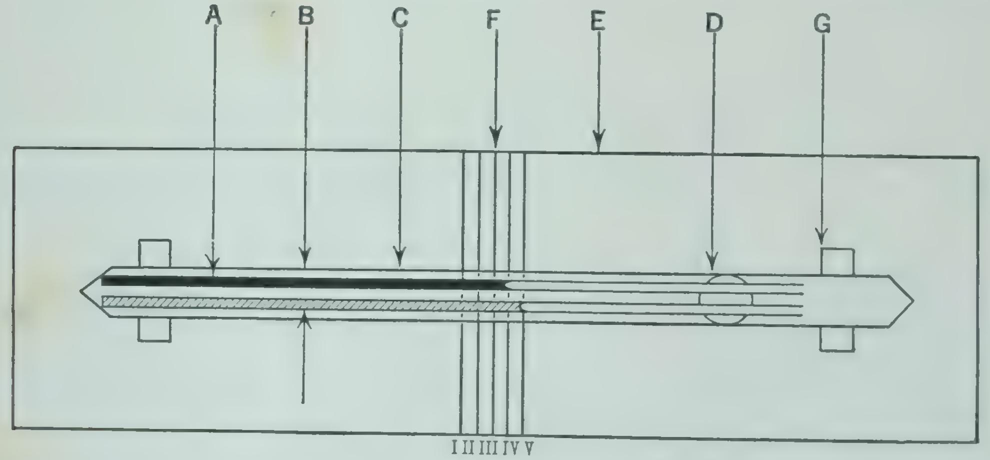


FIG. 56.

Glass Plate (Fig. 56*E*). A rectangular glass plate about 12 cm. long, 4 cm. wide, and 2 mm. thick which possesses five parallel transverse lines (F) situated near the center of the plate. The lines must not be more than 0.03 mm. thick and should be 3 mm. apart; they are appropriately marked (I, II, III, IV, V, etc.).

Capillaries. The capillaries are 10 to 12 cm. in length and about 1.5 mm. in inner diameter. They should be as uniform as possible, particularly in regard to the inner diameter (Fig. 56*A* and *B*).

Colored Glass Thread. Not more than 0.1 mm. in diameter.

Desiccator Tubes (Fig. 56*C*). These are ordinary glass tubings sealed at one end. They are 5 to 6 mm. in diameter and 15 cm. long.

Glass Wool (Fig. 56*D*). Pliable Pyrex glass wool is best, although ordinary cotton might be substituted.

Adhesive Tape (Fig. 56*C*). Ordinary surgical adhesive tape, 1 inch in diameter, is entirely satisfactory.

Volumetric Flasks with Ground-Glass Stoppers. Two 250-ml.; ½ dozen 100-, 50-, and 10-ml.; 1 dozen 25-ml.; and 1 dozen 1-ml. (Similar to the tare bottles for the absorption tubes, p. 34.)

Pipets. One-milliliter capacity, calibrated in 0.1 ml.

Hand Centrifuge. Ordinary, as used in clinical analyses. Equipped with two metal tubes. The capillaries are inserted directly into these metal tubes.

Vacuum Pump. An ordinary tap-water aspirator pump producing a vacuum of 20–40 mm. is adequate.

Reagents

Solvents. Low-boiling solvents which can be used under atmospheric pressure (acetone, chloroform, etc.) are preferable, but higher-boiling solvents (ethyl alcohol, benzene, pyridine, dioxane, etc.) can also be used. Since the solvent for the standard and the sample must be the same, a sufficient amount should be available not only for the preparation of the standard but also for the various unknowns to be examined.

Standard Solutions. Ten standard solutions: 0.2, 0.15, 0.1375, 0.125, 0.1125, 0.1, 0.0875, 0.075, 0.0625, and 0.05 molar. These standard solutions are best prepared as follows:

First 250 ml. of 0.2 M solution is prepared by dissolving an appropriate standard sample, preferably a colored substance, accurately weighed on a macrobalance, in a solvent suitable for both the standard and the test sample (acetone, chloroform, ethyl alcohol, pyridine, etc.). Exactly one-half of this standard solution is transferred to another 250-ml. volumetric flask, using a 100-ml. and a 25-ml. volumetric flask for the transfer. These two flasks are then rinsed several times with the original solvent and these rinsings are added to the solution followed by the addition of more solvent to bring the solution up to the mark. This then constitutes the 0.1 *M* standard solution. Twenty-five milliliters of the original 0.2 *M* solution is transferred into a 100-ml. volumetric flask and following the above rinsing and filling-up procedure, 100 ml. of the 0.05 *M* standard solution is obtained. Fifty milliliters of the same 0.2 *M* solution is transferred to another 100-ml. volumetric flask, but this time for the rinsing and the filling-up procedure portions of the 0.1 *M* solution are used to yield 100 ml. of the 0.15 *M* standard solution. Thus three more primary standard solutions (0.05, 0.1, and 0.15) are obtained.

The secondary standard solutions (0.0625, 0.075, 0.0875, 0.1125, 0.125, and 0.1375) are obtained by mixing equal quantities of the appropriate primary standard solutions, i.e.: 50 ml. of the 0.05 *M* solution is mixed with an equal amount of the 0.1 *M* solution to give 100 ml. of the 0.075 *M* standard solution. Twenty-five milliliters of this solution is again diluted with an equal amount of the 0.05 *M* primary standard solution to yield 50 ml. of the 0.0625 *M* standard solution; 25 ml. of the same 0.075 *M* solution is mixed with 25 ml. of the 0.1 *M* solution to form 50 ml. of the 0.0875 *M* standard solution. Fifty milliliters of the 0.15 *M* primary standard solution is introduced into a 100-ml. volumetric flask and, after rinsing of the 50-ml. transfer flask with portions of the 0.1 *M* solution and filling up the 100-ml. flask to the mark with portions of the same solution, 100 ml. of the 0.125 *M* standard solution is obtained. To prepare 50 ml. of the 0.1125 *M* solution, 25 ml. of the 0.1 *M* solution is mixed with an exactly equal amount of the 0.125 *M* solution, and finally 25 ml. of this solution is mixed with 25 ml. of the 0.15 *M* primary standard solution to yield 50 ml. of the 0.1375 *M* standard solution.

The ten standard solutions as prepared above are transferred and stored in properly labeled 25-ml. volumetric flasks.

Procedure

Preparation of the Sample Solution. The sample solution might be prepared on a macro (above 100 mg. substance), semi-micro (above 30 mg. substance), or micro scale. In the last case, 4 to 6 mg. of the sample is weighed in the weighing tube and transferred to a glass-stoppered tare bottle. This bottle with the substance is then weighed on an ordinary analytical balance within ±1 mg. After the addition by means of a dropping pipet of approximately 0.5 ml. of the identical solvent as used for the standard solution, the bottle is weighed again while stoppered. The weight of the solvent thus found is divided by the specific gravity of the solvent to give the volume.

Preparation of Capillaries (Fig. 56*A* and *B*). One of the capillaries is filled to two-thirds its length (i.e., 8 cm.) with the solution of the unknown (*B*) by means of suction by mouth. For very volatile or mobile liquids it is advisable to have that end which dips into the solution drawn out to a hair-fine ending. That end of the capillary which is opposite the liquid is sealed in a microburner. After cooling, the solution is centrifuged toward this sealed end by means of a suitable hand centrifuge. No violent centrifuging is necessary, a single turn of the handle usually suffices. Three more capillaries are then filled, sealed, and centrifuged in exactly the same manner. Similarly the set of capillaries (*A*) containing the standard solutions (0.2, 0.15, 0.1, and 0.5 molar) is prepared. If the standard solution is colorless, each capillary is marked by inserting a small colored glass thread (0.1 mm. thick and 2 mm. long) into it before centrifuging. After this, each standard solution capillary is paired off with a capillary of the unknown, preferably in such a way that the meniscuses of the two solutions (0.05 to 0.08 ml.) are at approximately the same level.

Filling the Desiccator Tube (Fig. 56). Each pair (*A* and *B*) is then introduced, sealed end down, into the desiccator tube (*C*). Both capillaries should rest firmly at the bottom of the tube. A wad of pliable glass wool (*D*) is then introduced and pushed down into the tube to about 5 mm. below the openings of the capillaries, but well above the meniscuses of the solutions. The purpose of this wad is to keep the capillaries in the same position. The lower half of the tube thus prepared is immersed in a beaker containing ice water. After ten minutes the tubes are removed and dried with a towel; if a solvent boiling below 70° C. is used, the tubes are sealed off as close as possible to the open ending of the capillaries. If a higher-boiling solvent, b.p. $> 70°$, is used, the part above the capillaries is softened in a gas flame. The walls of the tube are allowed to collapse, and this portion of the tube is then drawn out into a capillary without breaking off the constriction thus produced. The tube is then put back into the ice water and allowed to cool and is afterwards evacuated to about 30–40 mm. pressure. An ordinary tap-water aspirator pump suffices for this purpose. While the tube is still attached to the aspirator pump, the capillary constriction produced previously is once more heated in the gas flame and allowed to collapse completely, thus forming a seal. The desiccator tubes thus prepared are then appropriately marked and placed in the beaker containing ice water.

Mounting of the Desiccator Tubes. After sealing and cooling, the tube is mounted on the glass plate (*E*). The tube is first placed on the plate in such a way that the meniscuses of the two solutions contained in the enclosed capillaries come within the transverse markings (*F*). While the tube is held in this position, adhesive tape is applied at the two extreme ends and the tube is fastened to the plate. Two or three tubes may be mounted on the same plate. Both sides of the glass plate may be used.

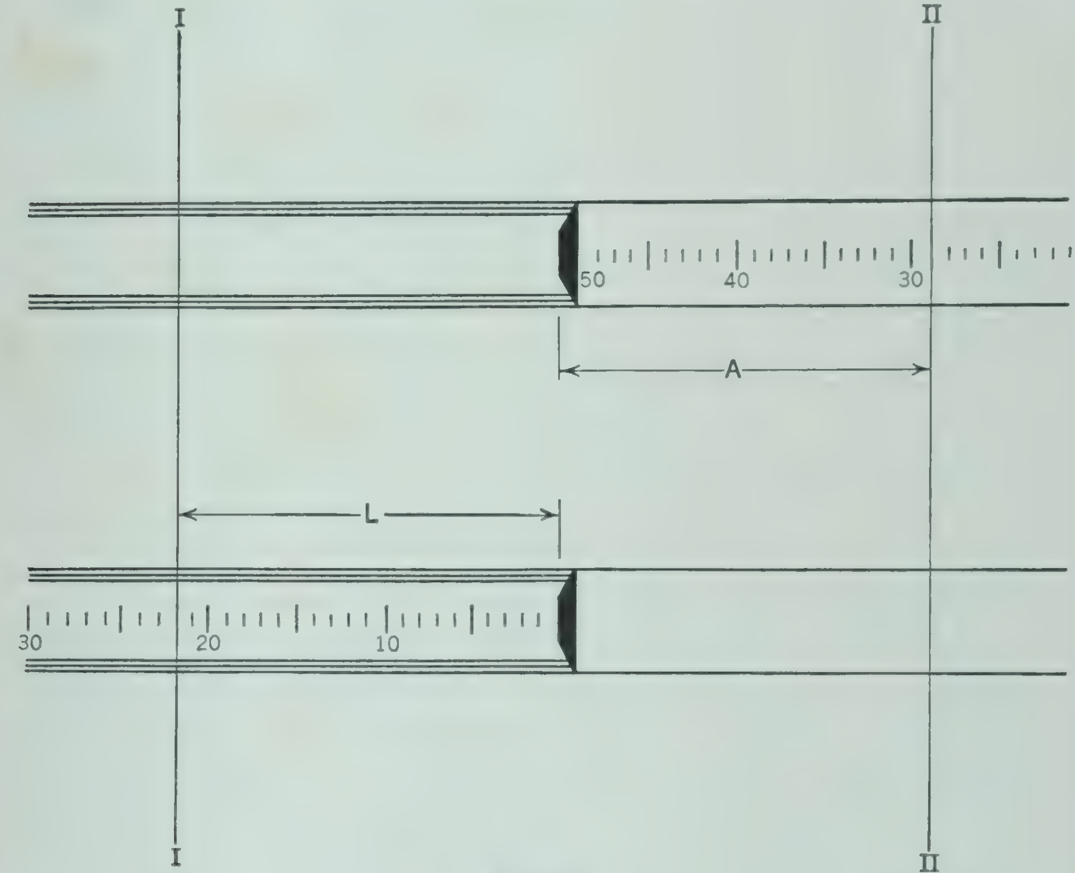


Fig. 57.

Method of Reading. Either the liquid column or the air column may be measured. The liquid column (*L*) is the distance of the solution between the apex of the meniscus and the nearest reference line situated within the column of liquid (Fig. 57*L*). The air column (*A*) is the distance between the apex of the meniscus and the nearest reference line above the liquid column (Fig. 57*A*). The reference lines (*F*) are distinguished by Roman numerals (I, II, III, IV, V).

Before the readings are taken under the microscope, the position of the meniscus of the standard solution in regard to the nearest reference line is noted (meniscus between reference line I and II [Fig. 57], etc.). After this, the plate with the mounted tubes is placed on the microscope stage with the liquid columns at the left. The meniscus of the standard solution is brought into focus, and then the nearest reference line is sought. Whether to take readings of the liquid or the air column is decided by whether the reference line within the micrometer scale reading is within the liquid column (L) or above the meniscus of the solution (A). If the liquid column is chosen, the microscope is focused at the apex of the meniscus (outermost point of the semicircular sharp shadow), and the plate moved until the zero mark of the micrometer scale coincides with this point. Then the microscope is focused upon the chosen reference line and the readings noted (Fig. 57, *LI* : 22).

If the air column is chosen, that is, when a reference line above the meniscus is within the micrometer scale range, again the microscope is focused upon the apex of the meniscus exactly the same as for the liquid column. The plate then is moved so that this point is at the number 50 of the micrometer scale. Without changing the position of the plate the microscope is now focused on the reference line and readings taken (Fig. 57, *AII* : 21).

Readings are repeated not oftener than every twenty-four hours in the beginning and about every two to three days thereafter for about two weeks. Usually, about five readings suffice. An increase in the liquid column is designated as plus (+), a decrease as minus (−). An increase in the air column is regarded as minus (−) and a decrease as plus (+). The results are then tabulated as shown below:

Micrometer Readings

	Standard Solution		*Unknown*	
Molarities	*1st*	*2nd*	*1st*	*2nd*
0.050	LII 40	LII 10	LIII 10	LIII 26
0.075	AIII 10	AIII 30	AIV 24	AIV 10
0.100	LII 30	LII 20	AIII 30	AIII 20
0.125	AIV 7	LIV 7	LIII 10	AIII 10
0.150	AIII 20	AIII 4	LII 20	AII 10

					Unknown *	
	Standard Δ	*Unknown* Δ	*Standard*	*Unknown*	$\frac{b-a}{2}$	$\frac{a+b}{2}$
0.050	−30	+16	−	+	+23	+7
0.075	−20	+14	−	+	+17	+3
0.100	−10	+10	0	0	0	0
0.125	+14	−20	+	−	−17	−3
0.150	+16	−30	+	−	−23	−7

* *For plotting:*

x: molarity of the standard solution;

y: micrometer units;

a: Δ standard solutions;

b: Δ unknown.

INTERPRETATION OF READINGS

From such a table the molarity at which a set of capillaries is *in* or *near* the *isopiestic state* and consequently of *equal* or *nearly equal molarity* can be ascertained in several ways. Such a set of capillaries may exhibit:

(*a*) *No Change.* The set in which both the standard solution and the solution of the unknown exhibit exactly the same decrease in volume, whereas the other sets in the series show differences.

(*b*) *Minimum Change.* The set in which there is the least change in volume between the standard solution and the solution of the unknown (B); or the set which exhibits the smallest change from the preceding or the succeeding sets in a series (B'). In the table above the 0.1 M standard solutions fits both cases (B, B'). In B the results may be plotted, particularly when the changes are all in one direction. The molarity of the unknown may thus be approximated.

(*c*) *Reversal.* When a series of sets is staggered according to increasing molarities as in the table above, the sets containing standard solutions of lower molarity than the unknown will have decreased in volume ($-$) while those containing standard solution of higher molarity than the solution of the unknown will show an increase ($+$) in volume of the standard solutions; the set between, at, or near the reversal from minus to plus if standard solutions are compared, or from plus to minus if the solution of the unknown is compared, is regarded as in or near the isopiestic state and consequently of equal or nearly equal molarity. In the table above this is clearly indicated in the 0.1 M standard solution.

The condition as given under (*a*) (no change) occurs very rarely. Thus it seems that the conditions at the surface of two equimolar solutions contained in two separate capillaries will seldom be identical and that difference in concentration of the surface layers may then be responsible for the ensuing volume changes.[14] Usually (*b*) (minimum change) is observed, particularly when the volumes of all solutions decrease, as happens during the early observations. It is therefore highly advisable to continue the readings, or to repeat the determination with standard solutions of different molarities, until a clear point of reversal as under (*c*) and as shown in the above table is observable.

Calculation:

Molecular weight: $\dfrac{c}{m}$

c: grams of sample in 1 liter of solvent;

m: molarity of the standard solution in the set which fulfills at least one of the conditions of isomolarity as given under (*a*), (*b*), or (*c*).

Remarks

The method described herein [5, 6] is a rigid application of the observations of the botanist L. Errara,[1] who found that the volumes of suspended droplets of salt solution in a closed system invariably increased, while those containing the solvent alone decreased. The method also follows closely the principle involved in the isothermic macromethod of R. A. Robinson and D. A. Sinclair [9] in which the loss or gain of solvent of two heteromolar solutions contained in separate containers, but placed in the same evacuated desiccator, is followed gravimetrically.

At the suggestion of L. Errara, G. Barger [1] developed his ingenious "microscopical method of determining molecular weight." In this method "a solution of known strength of the substance the molecular weight of which is unknown, is compared with standard solutions of a substance of known molecular weight, a series of drops taken alternately from the two solutions being introduced into a single capillary tube." The chief interference is from the mixing of the droplets either during the filling operation or on standing. The molarities used were from 0.1 to 0.25 M solutions, and an accuracy of $\pm 10\%$ is claimed. The method underwent several modifications notably by K. Rast,[8] who only used 1 drop of each of the two solutions and measured not the actual volume of the drops but the movement of the air bubble separating the two solutions. The molarities employed varied from 0.2 to 1 M solutions. A. Friedrich,[3, 4] in avoiding the rather high concentrations in the foregoing procedure, fixed the 2 drops by eliminating terminal air spaces, while J. R. Spiess [13] developed an improved method of filling the capillary. Further modifications involve the use of a U-shaped capillary,[2] actual determination of distillate,[11] and adaptation of the method on a macro scale.[12]

For student experiments a system involving a low-boiling solvent (acetone, chloroform, etc.) and allowing observation at atmospheric pressure is preferred. As a standard, azobenzene has been found extremely suitable. Azobenzene solutions of 0.05, 0.75, 0.1, 0.125, and 0.15 M are paired with a 0.1 M solution of the unknown in the same solvent. The present method possesses several advantages over G. Barger's and its subsequent modifications; e.g., the solutions cannot mix, the sample can be recovered, the changes in volume are much larger and permanent. The apparative arrangement and technic involved are of utmost simplicity.

LITERATURE

1. Barger, G., *J. Chem. Soc., Proceedings*, **19**, 121 (1903); *ibid., Transactions*, **85**, 286 (1904); *Ber.*, **37**, 1754 (1904).
2. Berl, E., and Hefter, O., *Ann.*, **478**, 235 (1930).
3. Friedrich, A., *Mikrochemie*, **6**, 97 (1928).
4. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Vienna and Leipzig. 1933, p. 191.
5. Niederl, J. B., and co-workers, *Science*, **92**, 225 (1940); **100**, 228 (1944).
6. Niederl, J. B., and Schmitt, R. B., *Bull. Am. Assoc. Jesuit Scientists*, **18**, 88 (1940).
7. Pregl, F., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, p. 296.
8. Rast, K., *Ber.*, **54**, 1979 (1921).
9. Robinson, R. A., and Sinclair, D. A., *J. Am. Chem. Soc.*, **56**, 1830 (1934).
10. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 244.
11. Schwarz, K., *Monatsh.*, **53**, 926 (1929).
12. Signer, R., *Ann.*, **478**, 246 (1930).
13. Spiess, J. R., *J. Am. Chem. Soc.*, **55**, 250 (1933).
14. West, Wm., New York University, New York, N. Y., private communication.

STRUCTURE ANALYSIS

I. DETERMINATION OF ALKOXYL AND ALKIMIDE GROUPS

ALKOXYL GROUPS

Principle

The organic compound containing one or more alkoxyl groups (ethers, esters, etc.) is refluxed with hydriodic acid, whereby the alkyl radical attached to oxygen is split off in the form of the corresponding alkyl iodide.[37] The resulting alkyl iodide is determined iodometrically.[9, 20, 33] The method is usually applicable to compounds containing *O*-methyl and *O*-ethyl groups.

$$(a)\quad \left.\begin{aligned} &\text{ROR} \xrightarrow[\Delta]{+2\text{HI}} 2\text{RI}\\ &\text{AROR} \xrightarrow[\Delta]{+\text{HI}} \text{RI}\\ &\text{RCO.OR} \xrightarrow[\Delta]{+\text{HI}} \text{RI} \end{aligned}\right\} \text{(hydrolysis)}$$

$$(b)\quad \text{RI} + \text{Br}_2 = \text{RBr} + \text{IBr}$$

$$(c)\quad \text{IBr} + 3\text{H}_2\text{O} + 2\text{Br}_2 = \text{HIO}_3 + 5\text{HBr} \text{ (absorption and oxidation)}$$

$$(d)\quad \text{HIO}_3 + 5\text{HI} = 3\text{I}_2 + 3\text{H}_2\text{O}$$

$$(e)\quad 3\text{I}_2 + 6\text{Na}_2\text{S}_2\text{O}_3 = 6\text{NaI} + 3\text{Na}_2\text{S}_4\text{O}_6 \text{ (titration)}$$

Apparatus

Alkoxyl Apparatus. The all-Pyrex-glass apparatus illustrated in Fig. 58 [9] consists of four parts: the reaction flask (*A*) with the washer and the side arm (*C*) the delivery tube with a spiral (*E*), and the receiver (*F*).

The reaction flask (*A*) is 2 cm. in diameter; its neck, which carries a standard ground-glass joint, is 6 cm. long and 8 mm. in outer diameter. The carbon dioxide inlet tube (*B*), which extends almost to the bottom of the reaction flask, is 7.5 cm. long and 5 mm. in diameter.

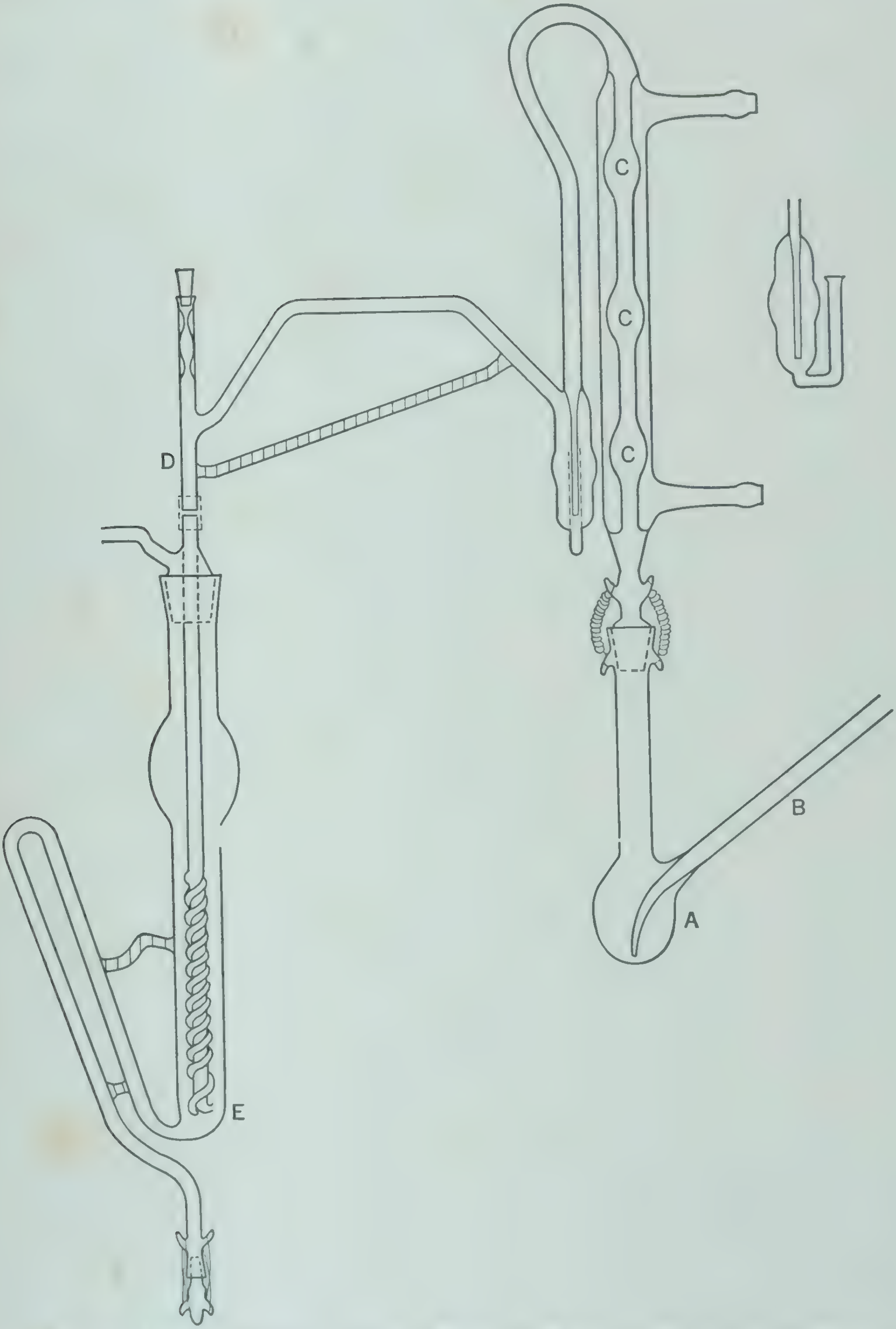


Fig. 58. Alkoxyl Apparatus. Inset: Sideview of Washer. Explanation in Text.

The vertical condenser (C) is 20 cm. long and 5 mm. in inner diameter; it carries a water jacket and three condensation bulbs. The washer, which is parallel to the condenser, is 5 cm. long and 1.2 cm. in diameter at its widest point. The washer is attached to the condenser by means of a suitably bent tube 5 mm. in diameter. The washer also carries a reinforced side arm of about 10-cm. length which terminates in the upper section of the vertical delivery tube. This section of the delivery tube, which is open at both ends, is 6.5 cm. long and 3 mm. in outer diameter. The detachable lower and longer portion of the delivery tube carries a spiral 9 cm. long and an outlet above its ground-glass joint. It is connected with the delivery tube of the apparatus by means of a piece of rubber tubing, forming a glass-to-glass connection. The upper portion of the receiver widens to 2 cm. and finally terminates in the ground-glass joint of the delivery tube of the apparatus. The bottom of the receiver ends in the siphon tube (G), which is 3 mm. in outer diameter. The ascending arm of the siphon is 9 cm. and the descending arm 11.5 cm. long. The descending arm terminates in a vertical section 3.5 cm. long, the outlet of which is provided with a ground-glass stopper placed exactly below the bottom of the receiver.

Kipp Generator. A Kipp generator or a carbon dioxide tank is a suitable source for the carbon dioxide, which is freed from possible halogen vapors by passing it through a gas wash bottle containing a saturated solution of sodium carbonate. A pinchcock is attached to the rubber tubing connecting the Kipp generator with the gas wash bottle to facilitate the regulation of the flow of carbon dioxide.

Glass-Stoppered Pyrex Glass Erlenmeyer Flasks (125-ml. capacity).

Buret. Either a microburet of 10-ml. capacity (p. 51), or still better a well-calibrated 50-ml. macroburet may be used for the 0.01 N sodium thiosulfate solution.

Reagents

Hydriodic Acid (sp. gr.: 1.7). Hydriodic acid should be fresh and colorless. All conditions which promote its decomposition and the separation of iodine caused by exposure to light and, more particularly, by access of air, should be avoided. It is preferably kept in an icebox.

Hydriodic Acid (sp. gr.: 1.96).

Phenol. C.P. crystals.

Propionic Acid Anhydride. C.P., boiling point: 64–66° C. at 15 mm. pressure.

Washer Solution. Suspension of red phosphorus in 5% aqueous cadmium sulfate solution.

Sodium Acetate in Glacial Acetic Acid (10%).

Bromine. Free from iodine.
Formic Acid (98 to 100%); melting point: 6 to 8° C.
Aqueous Sodium Acetate Solution (10%).
Sulfuric Acid Solution (10%).
0.01 *N Sodium Thiosulfate Solution* (p. 56).
Starch Indicator Solution (p. 54).

Procedure

Preparation of the Sample

Solids. Solid substances, previously dried and ground, are weighed in the weighing tube and placed on the bottom of the dry reaction flask. For semi-solids, or substances of syrupy consistency, and non-volatile liquids, a small glass cup, about 4 to 5 mm. in diameter and height, which fits conveniently into the neck of the reaction flask, is used. A piece of glass in the shape of a semicylindrical boat or trough with open ends, made from glass tubing of suitable diameter by cutting it lengthwise, or a piece of platinum foil molded to a boat-like shape, also serves the purpose.

Liquids. Volatile liquids are weighed in a small glass cup with a ground-glass stopper, or in a weighing pipet (p. 46). The weighed capillary pipet is centrifuged, cut just above the meniscus of the liquid, and both halves are then placed in the reaction flask.

Introduction of the Sample and Solubility Tests. A few crystals of phenol and 6 to 10 drops of propionic acid anhydride are added to the substance in the reaction flask, which is shaken to effect solution of the sample in the solvent. If the substance does not completely dissolve, the reaction flask is cautiously heated over a steam bath or over a very small flame to about 60 to 80° C. If complete solution still does not take place, a little more of the solvents is added until the substance has completely dissolved. Since this additional solvent will considerably lower the concentration of the hydriodic acid, a corresponding amount of hydriodic acid of sp. gr. 1.96 must be added to restore its original concentration. After allowing the solution to cool, a few Alundum beads of size 16 are placed in the reaction flask and then about 2 ml. of hydriodic acid (sp. gr.: 1.7) is added dropwise from a pipet while the flask is rotated to wash down any particles of substance that may adhere to the wall.

Preparation of the Apparatus and Heating. The washer of the apparatus is filled with 0.5 ml. of washer solution and stoppered with a cork. Then 4 to 5 ml. of glacial acetic acid-sodium acetate solution, to which 6 to 8 drops of bromine has been added, is placed into the receiver. The glass spiral is inserted into the solution, the reaction flask is connected as quickly as possible to the condenser, through which water is circulated, and the side arm is attached to the source of carbon dioxide, the flow of which is reduced to a slow stream. The flow of carbon dioxide is best adjusted by inserting a stopcock having a fine groove between the side arm and the carbon dioxide generator. The reaction flask is allowed to stand at room temperature for thirty minutes,[1] after which a small flame is placed under the flask and so regulated that the temperature of the solution remains below the boiling point for another thirty minutes. Then the flame is slowly raised and the solution is brought to boiling and maintained at that temperature for one hour or sometimes even longer (two to three hours). About fifteen minutes prior to the completion of the determination, the rate of carbon dioxide passing through the system is increased; the water in the condenser is allowed to drain off, and after about fifteen minutes the flame is removed.

Titration. The titration procedure [33] is the same as in the iodometric determination of iodine (p. 174). After completion of the heating and sweeping-out process the receiver is emptied by washing its contents into a ground-glass-stoppered Erlenmeyer flask which contains 5 ml. of aqueous sodium acetate solution. The excess of free bromine is destroyed by adding a few drops of formic acid while shaking the Erlenmeyer flask. The flask is allowed to stand for a few minutes, after which it is stoppered and shaken well to remove the last traces of bromine from the solution. If, after this treatment, the solution is not completely decolorized, another drop of formic acid is added and the flask is shaken again. Then 2 ml. of 10% potassium iodide solution is added and the solution is acidified with 3 ml. of 10% sulfuric acid; the flask is again stoppered and shaken. In the event of a high methoxyl value, which usually is evidenced by the intensity of the color of the liberated iodine, the time of standing is extended from the usual five minutes to fifteen minutes, before the solution is titrated with 0.01 *N* sodium thiosulfate solution.

Time:

	Minutes
Weighing of the sample	10
Preparation of the apparatus and introduction of the sample	30
Heating	120
Titration	15
Total	175

Calculation:

Log of ml. of 0.01 N sodium thiosulfate solution,
Plus log of factor,
Plus negative log of weight of sample;
Antilog of total = percentage of alkoxyl.

Factors:

Methoxyl: 0.05172; log, 71366.
Ethoxyl: 0.07510; log, 87564.

ALKIMIDE GROUPS

Principle

The organic compound containing one or more N-alkyl groups (primary, secondary, and tertiary amines, etc.) is first refluxed with hydriodic acid to form the respective quaternary alkyl ammonium iodide, which then is subjected to pyrolysis. The lower alkyl radicals (methyl, ethyl, etc.) are thus split off from the nitrogen in form of their alkyl iodides,[10, 14] which are then determined iodometrically.

(*a*) $>N-R + HI \rightarrow >N-R \cdot HI \xrightarrow{\Delta} RI$ (pyrolysis)

(*b*) $RI + Br_2 = RBr + IBr$

(*c*) $IBr + 3H_2O + 2Br_2 = HIO_3 + 5HBr$ (absorption and oxidation)

(*d*) $HIO_3 + 5HI = 3I_2 + 3H_2O$

(*e*) $3I_2 + 6Na_2S_2O_3 = 6NaI + 3Na_2S_4O_6$ (titration)

Apparatus

The determination[10a–d] is carried out in an apparatus made of Pyrex glass (Fig. 59). It consists of an olive-shaped reaction flask (*a*) of about 5-ml. capacity, which has a side inlet (*b*) 12 cm. long and 6 mm. in diameter for the introduction of the sample and the carbon dioxide. The delivery tube (*c*) is 12 to 13 cm. long and about 6 mm. in diameter. It is fused to the reaction flask in a position to form an angle of about 45 degrees with the side inlet. The delivery tube continues in an almost horizontal line for about 8 cm. and then it is bent downward to extend vertically for 11 cm., when it is again bent horizontally for a length of 7 cm.; 2.5 cm. above this bend the vertical portion (*d*) of the right side arm (*e*) of the three-way stopcock is fused on. The delivery tube is then bent upward and at the same time widened to form the condensation bulb (*f*), which is 1.5 cm. in diameter and 3 cm.

long. The extension of the delivery tube connects this condensation bulb with the horizontal left side arm (e') of the three-way stopcock. Thus the two side arms of the stopcock and the delivery tube form a rectangle, the lower portion of which is heated during the determination by immersion in hot water. Beyond the connection of the delivery tube with the left arm of the stopcock the delivery tube continues horizontally for about 5 cm., after which it is again bent downward at right angles and extends into the washer (g) as a capillary of about 1-mm. inner diameter. The side arm of the washer is connected to the vertical delivery tube (h), which is 6.5 cm. in length, 3 mm. in diameter, and open at both ends.

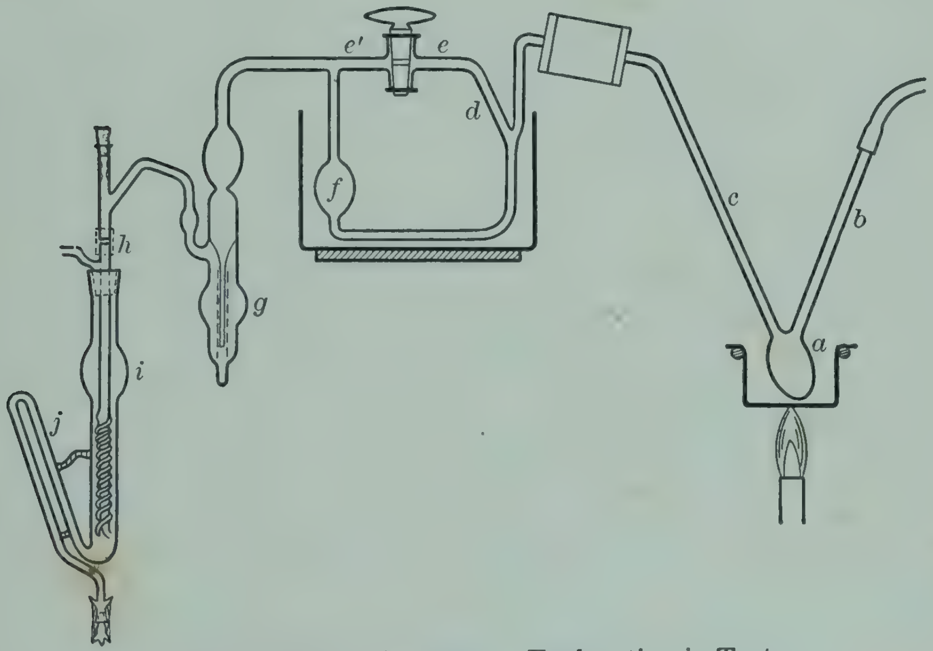


Fig. 59. Alkimide Apparatus. Explanation in Text.

The detachable lower and longer portion of the delivery tube (i) carrying a spiral, and the siphon-receiver (j), as well as all other apparatus, such as the Kipp generator, Erlenmeyer flasks, and titration equipment, are the same as in the alkoxyl determination (p. 241).

Reagents

Aside from the reagents listed in the alkoxyl determination the following two reagents are necessary:

Aqueous Gold Chloride Solution (5%).
Ammonium Iodide. C.P.

Procedure

Preparation of the Sample and Apparatus. The preparation of the sample, including the solubility tests, and the cleaning and drying of the alkimide apparatus are the same as in the alkoxyl determination. After the washer has been filled with 0.5 ml. of 5% sodium thiosulfate solution, the substance is introduced into the reaction flask and is dissolved in the phenol-propionic anhydride mixture; a spatula point of ammonium iodide is added, and then 1 or 2 drops of gold chloride solution and 2 ml. of hydriodic acid. The receiver is filled with 4 to 5 ml. of glacial acetic acid-sodium acetate solution, to which 6 to 8 drops of bromine has been added. After insertion of the spiral of the delivery tube into the receiver, and with the pinchcock between the inlet tube and the source of carbon dioxide closed, the rubber tubing is drawn over the side inlet tube, into which a small sealed tube has been inserted. The stopcock is opened and the current of carbon dioxide is regulated so that no more than 2 bubbles at a time rise in the receiver.

Heating. The reaction flask is placed in a metal pan containing powdered copper oxide and a thermometer. The lower portion of the rectangle is immersed in a dish of water which is heated to 90° C. The pan is heated very slowly to a temperature of 360° C.; this gradual heating should require about one hour. The heating at 360° C. is continued until all the hydriodic acid has been distilled off, whereupon the flame is extinguished and the apparatus is allowed to cool to room temperature. The stopcock is then turned crosswise, so that the vertical bore faces the washer (*g*). Next, the flow of the carbon dioxide is shut off, the rubber tubing is removed from the inlet tube, and the hydriodic acid in the lower part of the rectangle is siphoned back into the reaction flask by applying suction by mouth at this tube. About 0.5 ml. of fresh hydriodic acid is introduced through the vertical bore of the stopcock and is also siphoned back. The receiver is then exchanged for another one which has been freshly filled, and the stopcock is again turned to its original position. Carbon dioxide is passed through the apparatus and the heating is repeated at least once more as described above.

Titration. The titrations are carried out in exactly the same manner as described in the alkoxyl determination (p. 243).

Calculation:

Log of ml. of 0.01 *N* sodium thiosulfate solution,
Plus log of factor,
Plus negative log of weight of sample;
Antilog of total = percentage of alkimide.

Factors:

Methyl, 0.02503; log, 39863.
Ethyl, 0.04839; log, 68476.

Remarks

O-Alkyl

Iodometric Methods. The iodometric determination of *O*-alkyl groups as presented in this manual is the method of A. Elek,[9] which incorporates several improvements over the gravimetric method as devised by F. Pregl [28, 30] and the iodometric method of F. Vieböck and co-workers.[33] Both the gravimetric and the iodometric methods have been the subject of thorough investigation.[1, 2, 4, 6, 8, 11, 13, 14, 20, 23, 24, 31, 35]

The original Zeisel methoxyl macromethod,[37] as adapted and modified for microdeterminations by F. Pregl,[28, 30] consisted in heating the substance with hydriodic acid, driving off the alkyl iodide with carbon dioxide, absorbing it in alcoholic silver nitrate solution, and determining the silver iodide gravimetrically. Later F. Vieböck and co-workers [33] shortened the last part of the procedure by iodometric determination of the alkyl iodide distilling over.

The modified apparatus of D. R. Rigakos [29] consists in the introduction of a ground-glass joint between the reaction flask and the outlet tube, and of a capillary leading from the side arm near the bottom of the reaction flask. The same modification of the apparatus was described several years later by F. Neumann [25] for highly methylated carbohydrates. E. P. Clark [6] has described an apparatus having ground-glass joints and a capillary side arm, which can be used for macro as well as microdeterminations. J. J. Chinoy [5] has also constructed a modified apparatus with a ground-glass joint fitting into the reaction flask and with an extension ending in a glass ladle, or cup, which facilitates the introduction of the sample. These improvements have been applied to solid and syrupy substances and to liquids whose boiling points are above that of hydriodic acid.

A. F. Colson [7] has devised an apparatus for liquids having boiling points lower than that of the hydriodic acid. This apparatus consists of a vertical tube connected above the reaction flask; the tube is filled with small glass beads moistened with hydriodic acid. Two washing tubes are connected with the train to entrap any hydriodic acid that might have distilled over; hydriodic acid of sp. gr. 1.96 is used.

M. Lieff and co-workers [22] have described a modified apparatus which is stated to be more practical for the iodometric method. The Pregl type of receiver is replaced by an absorption tube, containing four *pockets,* which is attached to the outlet tube of the Pregl type of apparatus by a ground-glass joint in an inclined position. A novel type of receiver has been described by B. E. Christensen and co-workers.[8]

In the determination of alkoxyl groups it is important that there be thorough absorption, and in the titrimetric method complete oxidation of the alkyl iodide to iodate. This is achieved by means of a long and slow passage of the alkyl iodide through the absorption liquid. For this purpose a glass spiral, consisting of about twelve turns, was devised by A. Elek.[9] It is attached to the outlet tube, which is immersed in the absorbent contained in a modified L. Kahovec [15] type of receiver.

A. Friedrich [10] states that the method is quite accurate if the hydriodic acid is pure, and that the substance should be brought into solution before the addition of the hydriodic acid. G. M. Ware [34] reports that, if the substance contains more than two methoxyl groups, the hydriodic acid of sp. gr. 1.7 should be replaced by hydriodic acid of sp. gr. 1.96 in order to obtain reliable results. For highly methylated carbohydrates F. Arndt [1] and F. Neumann [25] mention that methoxyl values which are 1 to 2% low have often been reported; they attribute this discrepancy to the fact that the reaction flask is heated over a free flame and that as a consequence the substance in the flask is more or less *caked*. According to them, this caked portion of the substance does not react with the hydriodic acid, and the variation in the degree of caking is responsible for the non-reproducibility of values obtained from the same sample, especially for polymeric carbohydrates. Therefore, an oil bath with gradual elevation of the temperature is used to circumvent this detrimental effect.

V. Bruckner [4] obtained correct results in some cases without the use of any solvent. F. Pregl [28, 30] and others [18] mention separate solubility tests before the addition of hydriodic acid, but these are obviously not feasible when only a small amount of substance is available.

Gravimetric Methods.[10, 28, 30, 34, 36] The iodometric method of A. Elek,[9] as described in this manual, may also be used for the gravimetric determination of alkoxyl groups in organic compounds, particularly for the occasional checking of the iodometric method. In this method the siphon of the receiver (Fig. 58*F*) is omitted, and a 4% alcoholic silver nitrate solution is used as the absorbing agent.

$$\mathrm{RI} + 3\mathrm{AgNO_3} \rightarrow \mathrm{AgI \cdot 2AgNO_3} \xrightarrow[\mathrm{H_2O}]{\Delta} \mathrm{AgI}$$

The procedure remains the same. After completion of the heating and sweeping-out, the apparatus is raised sufficiently so that the tip of the delivery tube is about 2 cm. above the solution in the receiver. Next, the cork is removed from the top and the delivery tube is rinsed from above, inside and outside, with dilute nitric acid and alcohol. Should particles of silver iodide still adhere to the delivery tube, it is again rinsed alternately with these reagents. Mechanical removal with the aid of a feather or rubber policeman should be resorted to only after attempts to remove the last particles by rinsing have failed. Five drops of concentrated nitric acid is added to the solution in the receiver and the receiver is heated on the steam bath until the contents begin to boil. Then the receiver is cooled under the tap and when cool the silver iodide precipitate is filtered with a halogen filter tube; the precipitate is washed, dried, and weighed as described in the halogen determination on p. 157.

An empirical correction of 0.06 mg. silver iodide for each milliliter of alcoholic silver nitrate solution must be added to correct for incomplete precipitation of the alkyl iodide.[10]

Calculation:

Log of weight of precipitate,
Plus log of factor,
Plus negative log of weight of sample;
Antilog of total = percentage of methoxyl or ethoxyl.

Factors:

Methoxyl, 0.1321; log, 12096.
Ethoxyl, 0.1918; log, 28287.

S-Alkyl. The possibility of applying the conventional semi-micro [6] and micro alkoxyl [33] methods for the determination of *S*-alkyl groups (thio ethers) has been investigated repeatedly. Satisfactory [9, 28, 30] as well as unsatisfactory [1, 3] results have been reported. Usually a longer heating period (three to eight hours) is necessary.

N-Alkyl.[10, 11, 28, 30] The alkylimide method described in this manual is a modification of the method devised by A. Friedrich.[10] Great caution has to be exercised in interpreting the results. Some substances yield only one-half or one-third of their number of *N*-alkyl radicals, such as certain alkaloids and complex *N*-heterocyclic compounds.

Hydriodic Acid.[8, 10, 28, 30, 32] Most important in any of the foregoing O-, S-, or N-alkyl determinations is the quality of the hydriodic acid (sp. gr.: 1.7) employed. Several suggestions for its preparation and purification have been made.[6, 10] According to A. Friedrich [10] the purification and regeneration of this acid are best accomplished by refluxing commercial hydriodic acid with red phosphorus until complete decolorization has taken place. The acid is then distilled in an all-glass apparatus in an atmosphere of nitrogen. E. P. Clark [6] suggests the following method for the preparation of the acid: 254 grams of iodine and 185 grams of water are heated to about 50° C. in a 500-ml. flask having a ground-glass condenser, and 66 grams of 50% hypophosphorous acid (H_3PO_2), which must be free from sulfate, is added in portions at such a rate that the mixture is boiling continuously until the iodine is reduced. Heat is then applied to the flask and the boiling is continued for three hours, during which time a current of carbon dioxide is passed through the solution. The position of the condenser is changed to allow distillation, and the constant-boiling hydriodic acid is collected. The yield is 447 grams. The hydriodic acid thus prepared is stored in dark bottles and preserved by the addition of a little 50% solution of hypophosphorous acid (about 1 ml. per 450 grams).

Under some conditions, for instance, in the determination of alcohols or of glycerol in aqueous solutions, or when too large an amount of solvent had to be used, it is necessary to employ hydriodic acid of sp. gr. 1.96 in order to maintain and retain a specific gravity of 1.7 in the reaction mixture.

OTHER METHODS

Methoxyl and *ethoxyl* groups may also be determined *simultaneously*. The alkyl iodides as formed by the hydrolysis with hydriodic acid in the usual manner are collected in an alcoholic solution of *trimethylamine*; thus tetramethyl ammonium iodide and trimethylethyl ammonium iodide are formed.[19] Since the former is almost insoluble in absolute alcohol and the latter is readily soluble, the separation of the salts is possible.[36] After the addition of dilute nitric acid and silver nitrate to the separated salts, the iodine is precipitated and determined gravimetrically as silver iodide.

Pyridine may also be used for the absorption of alkyl iodides,[17, 21, 23] both for qualitative [23] and for quantitative determinations. Application of the original micro alkoxyl determination method as devised by F. Pregl [28, 30] to the *quantitative determination* of minute amounts of *ethyl alcohol* in aqueous solutions (steam distillates of human brains) has been reported by A. O. Gettler, J. B. Niederl, and A. A. Benedetti-Pichler.[12, 26]

The alkyl iodides, as obtained in *O*-, *S*-, and *N*-alkyl determinations, may be identified as 3,5-dinitrobenzoates, or as α-naphthylamine derivatives.[11]

Applying appropriate modifications to the conventional micro Zeisel apparatus, J. B. Niederl and M. Brenner [27] succeeded in the conclusive identification of *ethylene* as given off by ripening fruits, thus indicating that such an apparatus is a valuable research instrument in certain isolation and identification procedures.[12, 26, 27]

LITERATURE

1. ARNDT, F., and co-workers, *Ann.*, **510**, 71 (1934); *Ber.*, **70B**, 1835 (1937); **72B**, 1860 (1939).

2. Auwers, K., and co-workers, *Ber.*, **42**, 544 (1909).
3. Baernstein, H. D., *J. Biol. Chem.*, **97**, 663 (1932); **106**, 451 (1934); **115**, 25 (1936).
4. Bruckner, V., *Mikrochemie*, **12**, 153 (1932).
5. Chinoy, J. J., *Analyst*, **61**, 602 (1936).
6. Clark, E. P., *Ind. Eng. Chem., Anal. Ed.*, **10**, 677 (1938); *J. Assoc. Official Agr. Chem.*, **15**, 136 (1932); **22**, 622 (1939).
7. Colson, A. F., *Analyst*, **58**, 594 (1933).
8. Christensen, B. E., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **8**, 194 (1936); **13**, 276 (1941).
9. Elek, A., *Ind. Eng. Chem., Anal. Ed.*, **11**, 174 (1939).
10. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Vienna and Leipzig, 1933; (*a*) pp. 133–160, (*b*) pp. 151–159; (*c*) *Z. physiol. Chem.*, **163**, 141 (1927); (*d*) *Mikrochemie*, **7**, 185, 195 (1929).
11. Furter, M., *Helv. Chim. Acta*, **21**, 872, 1144, 1151 (1938).
12. Gettler, A. O., Niederl, J. B., and Benedetti-Pichler, A. A., *J. Am. Chem. Soc.*, **54**, 1476 (1932); *Z. angew. Chem.*, **44**, 457 (1931); *Mikrochemie*, **11**, 167 (1932).
13. Gibson, D. T., and Caulfield, T. H., *J. Chem. Soc.*, **1935**, p. 1419.
14. Herzig, T., and Meyer, H., *Ber.*, **27**, 319 (1894); *Monatsh.*, **15**, 613 (1894); **16**, 599 (1895); **18**, 379 (1897).
15. Kahovec, L., *Mikrochemie*, **14**, 341 (1934).
16. Kinsman, S., and Noller, C. R., *Ind. Eng. Chem., Anal. Ed.*, **10**, 424 (1938).
17. Kirpal, A., and Buhn, T., *Monatsh.*, **36**, 853 (1915).
18. Kuhn, R., and co-workers, *Ber.*, **67**, 1458 (1934); **68**, 387 (1935).
19. Künster, W., and Maag, W., *Z. physiol. Chem.*, **127**, 190 (1923).
20. Leipert, T., *Mikrochemie, Pregl Festschrift*, 1929, p. 266.
21. Lieb, H., see reference 30, p. 179.
22. Lieff, M., and co-workers, *Can. J. Research*, **15**, 529 (1937).
23. Lisle, E. B., *Analyst*, **64**, 876 (1939).
24. Nanji, H. R., *Analyst*, **59**, 96 (1934).
25. Neumann, F., *Ber.*, **70**, 734 (1937).
26. Niederl, J. B., and Whitman, J. B., *Z. anal. Chem.*, **86**, 65 (1931).
27. Niederl, J. B., and Brenner, M., *Mikrochemie*, **24**, 134 (1938); *Am. J. Botany*, **25**, 357 (1938).
28. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, pp. 198–215.
29. Rigakos, D. R., *J. Am. Chem. Soc.*, **53**, 3903 (1931).
30. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 171–191.
31. Slotta, K. H., and Haberland, G., *Ber.*, **65**, 127 (1932); *Mikrochemie*, **11**, 157 (1932).
32. Stoll, A., and co-workers, *Helv. Chim. Acta*, **17**, 654 (1934).
33. Vieböck, F., and co-workers, *Ber.*, **63**, 2818, 3207 (1930); *Mikrochemie*, **10**, 188 (1932).
34. Ware, G. M., *Mikrochemie*, **8**, 352 (1930).
35. White, E. V., and Wright, G. F., *Can. J. Research*, **14**, 427 (1936).
36. Willstätter, R., and Utzinger, M., *Ann.*, **282**, 148 (1911).
37. Zeisel, S., *Monatsh.*, **6**, 989 (1885).

II. DETERMINATION OF ACYL GROUPS

ALKALIMETRIC METHOD

Principle

The organic substance containing *O*-acyl or *N*-acyl groups (esters, amides, etc.) is hydrolyzed in either an acidic or alkaline medium, depending upon the solubility of the substance. After hydrolysis the volatile acidic hydrolysis products are subjected to steam distillation and the distillate is collected in several portions. The distillates are then titrated separately with 0.01 *N* sodium hydroxide solution, and from the total amount of standard alkali consumed the percentage acyl is calculated.[10, 17, 19]

$$(a)\quad \mathrm{RCOOR} \xrightarrow{\text{hydrolysis}} \mathrm{ROH} + \mathrm{RCOOH}$$

$$(a')\quad \mathrm{RCONR_2} \xrightarrow{\text{hydrolysis}} \mathrm{NR_2H} + \mathrm{RCOOH}$$

$$(b)\quad \mathrm{RCOOH} + \mathrm{NaOH} = \mathrm{RCOONa} + \mathrm{H_2O} \quad \text{(titration)}$$

Apparatus

Bubble Counter and U-Tube. These are the same as used in the carbon and hydrogen determination (p. 104). The bubble counter is filled with 50% potassium hydroxide solution and the U-tube with Ascarite.

Hydrolysis Apparatus (Fig. 60). This is an all-Pyrex glass or quartz apparatus consisting of the three-necked reaction flask, the gas inlet tube, the funnel, and the condenser. The *reaction flask* has a capacity of 60 ml. and consists of the reaction chamber, which is 5 cm. in diameter and 4.5 cm. in height, and has three necks. The neck at the left, 9.5 cm. long and 7 mm. in outer diameter, serves for the insertion of the gas inlet tube, the descending portion of which is 18 cm. and the horizontal portion 6 cm. long. This *inlet tube* carries on the outside a ground-glass joint which fits into the neck at the left. The center neck is also 9.5 cm. long, but is 1.2 cm. in outer diameter; it is provided with an outer ground-glass joint into which fits the inner ground-glass joint of the funnel. The *funnel* has an overall length of 16 cm.; the upper portion has a capacity of 8 ml. and is 8.5 cm. long and 1.5 cm. wide; the stem is 8 cm. long and 4 mm. in diameter. The funnel carries two ground-glass joints, of which the upper fits into the neck of the reaction flask, the lower one inside serving as a seal for the *plunger;* the plunger is 12 cm. long and 4 mm. in diameter and is provided with an inner ground-glass joint. The *condenser tube* is 50 cm. long and 8 mm. in outer diameter and carries a water jacket 30 cm. long and 2.2 cm. in outer diameter. The condenser tube terminates on one end in an outlet 7 cm. long and 8 mm. in diameter and bent at 135 degrees. This outlet tube is provided with a ground-glass joint which fits into the neck at the right of the reaction flask. The opposite end of the condenser tube terminates in a similar outlet tube, which is bent in the same direction, but only at 90 degrees. Thus the condenser can serve either as a vertical reflux condenser or as an inclined water condenser. All ground-glass joints carry glass hooks for the attachment of steel springs.

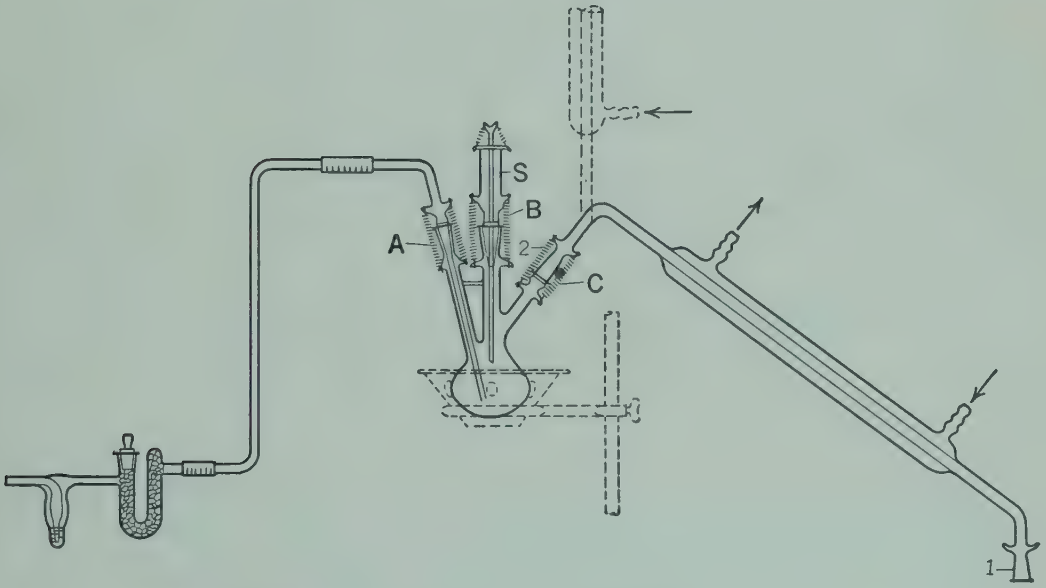


FIG. 60. Acyl Apparatus. Explanation in Text.

Water Bath. Either a metal bath or a 1-liter beaker can be employed.

Pipets. Of 0.5, 1.5 and 10-ml. capacity; or a 10-ml. pipet graduated in 0.1 ml.

Titration Equipment. The same as described under "Standard Solutions" (p. 51).

Reagents

Aqueous Sodium Hydroxide Solutions: 1 *N* and 5 *N*.

Alcoholic Sodium Hydroxide Solution. Four grams of sodium hydroxide pellets is dissolved in 50 ml. of distilled water; the resulting solution is mixed with 50 ml. of methyl alcohol previously refluxed and redistilled over solid potassium hydroxide.

Concentrated Sulfuric Acid (sp. gr.: 1.84).

Dilute Sulfuric Acid. To 200 ml. of distilled water is added 100 ml. of concentrated sulfuric acid.

Sulfonic Acid Solution. Twenty-five grams of *p*-toluene sulfonic acid is dissolved in 100 ml. of distilled water.

Metaphosphoric Acid. This acid is used as a lubricant for the ground-glass joints. It is prepared by the addition of small amounts of water to phosphorus pentoxide.

Pyridine. C.P.

Barium Chloride. Crystals, reagent quality.

0.01 *N Sodium Hydroxide Solution* (p. 56).

0.01 *N Acid Solution.* Either potassium biiodate or hydrochloric acid solution may be used (p. 54).

Phenolphthalein Indicator Solution (1%; p. 54).

Procedure

Solubility Tests. In order to select the proper hydrolyzing agent, the substance is first tested for its solubility in the four principal hydrolyzing agents, i.e., aqueous sodium hydroxide solution, alcoholic sodium hydroxide solution, or dilute sulfuric and sulfonic acid solutions. The hydrolyzing agent in which the substance is most readily soluble is then chosen for the hydrolysis of the substance in question. Should the substance be insoluble in all four reagents, pyridine may be used as additional solvent.

Preparation of the Sample. Solids are weighed and introduced through the center neck into the reaction flask of the hydrolysis apparatus by means of the weighing tube. Volatile *liquids* are weighed in the usual weighing pipets, while less volatile liquids, semi-solids, and syrups are weighed in capillaries open at both ends, one end of which may be drawn out to a fine capillary. The filled and weighed capillary is cut above the meniscus of the liquid, and both bulb and stem are thrown into the reaction chamber of the hydrolysis apparatus which, with the condenser and inlet tube already attached, has been made ready for the determination.

Preparation of the Apparatus. The apparatus must be clean and dry before use. The bubble counter is connected to an oxygen or nitrogen tank and the speed of the bubbles is adjusted to 1 bubble per second. The reaction flask is connected to the condenser, which is now in a vertical position, and then the gas inlet tube is inserted into the neck at the left of the reaction flask. The ground-glass joints are previously lubricated with metaphosphoric acid. Next, the hydrolyzing agent is added to the substance in the reaction flask through the center neck. (If the substance is volatile the hydrolyzing agent is introduced first and the capillary with the substance afterwards.) The following amounts of hydrolizing agent are used:

Acid Hydrolysis: 1 ml. *Alkaline Hydrolysis*: 1 ml. aqueous or 4 ml. of the alcoholic sodium hydroxide solution. After the addition of the hydrolyzing agent, the funnel, without the plunger and with its ground-glass joint on the outside properly lubricated, is inserted into the center neck and the gas inlet tube is connected to the bubble counter. Then the plunger is inserted into the upper portion of the funnel and a seal is formed by the introduction of 1 ml. of water into the upper portion of the funnel.

Hydrolysis. The reaction flask is completely immersed in the water bath and the water is brought to boiling; the heating is continued for twenty minutes, after which the water bath is removed and the apparatus is allowed to cool. The inside of the condenser is rinsed with 5 ml. of distilled water, which is allowed to drain into the reaction flask and then the condenser is removed, washed with 100 ml. of distilled water, and reconnected to the neck at the right with the wide-angle outlet tube, so that the condenser is inclined downward, as shown in Fig. 60. The direction of the water circulating through the cooling jacket is also changed accordingly. (If the hydrolysis was performed with alcoholic sodium hydroxide solution, the alcohol—4 ml. of distillate—is now distilled off. After the removal of the alcohol the condenser is again rinsed and washed with distilled water as described above.)

Distillation. After re-attachment of the condenser so that it again faces downward, the following reagent is introduced into the upper portion of the funnel:

(*a*) Sulfuric acid hydrolysis: 1 ml. of 5 N sodium hydroxide solution.
(*b*) Sulfonic acid hydrolysis: 0.5 ml. 1 N sodium hydroxide solution.
(*c*) Alkaline hydrolysis (either aqueous or alcoholic): 1 ml. dilute sulfuric acid.

The plunger is then lifted sufficiently to allow the reagent to flow into the reaction flask; this is followed by rinsing of the funnel with 2 ml. of distilled water, after which several pieces of Alundum are added to the reaction mixture; the plunger is re-inserted and 7 ml. of distilled water is introduced into the upper portion of the funnel. Using a small gas flame, distillation is started at a rate so that about 1 ml. per minute distils into the receiver, which may be a steamed-out and marked 125-ml. Erlenmeyer flask of Pyrex glass, or a similarly treated Pyrex glass graduate cylinder with a funnel. When the volume of the reaction flask has decreased to about 2 to 3 ml., more distilled water is slowly added, without interrupting the distillation, by lifting the plunger and allowing the distilled water in the funnel to run into the reaction flask. The funnel is again filled with 7 ml. of water and the above procedure is repeated until the total amount of the distillate collected in the receiver has reached a volume of about 20 ml. The receiver is then exchanged for another one, and this time a total of 15 ml. distillate is collected. This is repeated twice, about 5 ml. being collected each time, to give a total of four fractions of distillate: first, 20 ml.; second, 15 ml.; third and fourth, 5 ml. each. The time required for the distillation of the four fractions is about forty minutes.

Titration. The four distillates should be titrated separately. The first and the second may be titrated while distillation is still in progress. A few milligrams of barium chloride are added to the distillate, which is then quickly heated to boiling; no turbidity must occur. A few drops of indicator solution are added and the hot solution is titrated with 0.01 N sodium hydroxide solution to a faint pink coloration (see p. 67). The remaining distillates are titrated in exactly the same manner.

Calculation:

Log of total ml. of 0.01 N sodium hydroxide solution (sum of all four titrations),
Plus log of factor,
Plus negative log of weight of sample;
Antilog of total = percentage acyl.

Factors:

Acetyl ($CH_3CO—$): 0.4302; log, 63367.
Benzoyl ($C_6H_5CO—$): 1.0504; log, 02135.

IODOMETRIC METHOD

Principle

The organic substance containing *O*- or *N*-acetyl groups is hydrolyzed by means of *p*-toluenesulfonic acid in a closed system. The acetic acid formed after steam distillation is determination iodometrically.[4]

(*a*) $CH_3COOR \xrightarrow{CH_3C_6H_4SO_3H} ROH + CH_3COOH$

(hydrolysis)

(*b*) $CH_3CONR_2 \xrightarrow{CH_3C_6H_4SO_3H} NR_2H \cdot C_7H_7SO_3H + CH_3COOH$

(*c*) $6CH_3COOH + 5KI + KIO_3 = 3I_2 + 3H_2O + 6CH_3COOK$ (titration)

Apparatus

Acetyl Apparatus (Fig. 61). This consists of a distilling flask (*a*) to which a suitably constructed all-glass dropping funnel (*b*) is connected by a ground-glass joint. The ascending side arm (*c*) of the distillation flask is provided with a still head, and its downward section is connected to the inner tube of a water condenser, through which cold water is circulated during the distillation. Attached to this condenser is the receiver (*d*), which is provided with a suitable side arm for attachment to the vacuum pump. The apparatus is constructed wholly of Pyrex glass, the joints being especially ground to make them vacuum tight with only water as lubricant. The connection between the side arm of the flask and the condenser is made glass-to-glass and sheathed in steamed rubber tubing in order to provide sufficient flexibility to permit tapping of the flask. It is important that these parts be made of glass tubing having the same outside diameter as the side arm of the flask to facilitate air-tight connections.

Titration Equipment. See "Standard Solutions" (p. 51).

Reagents

Sulfonic Acid Solution (25%); (p. 255).

Potassium Iodide. C.P. crystals.

Potassium Iodate Solution (4%). Four grams of potassium iodate, of reagent purity, is dissolved in 100 ml. freshly boiled distilled water.

0.01 *N Iodine Solution.* This is prepared by diluting a 0.1 *N* iodine solution, or by dissolving 1.27 g. of analytical reagent iodine in a solution of an equal weight of analytical reagent potassium iodide in freshly boiled distilled water and diluting to 1 liter.

0.01 *N Sodium Thiosulfate Solution* (p. 56).

Starch Indicator Solution (1%) (p. 54).

Procedure

Preparation of the Apparatus. The entire apparatus, with the exception of the rubber connections, which are well steamed, is thoroughly cleaned with cleaning solution, well rinsed, and dried. Between successive determinations thorough rinsing with distilled water is sufficient and only the distilling flask need be dried.

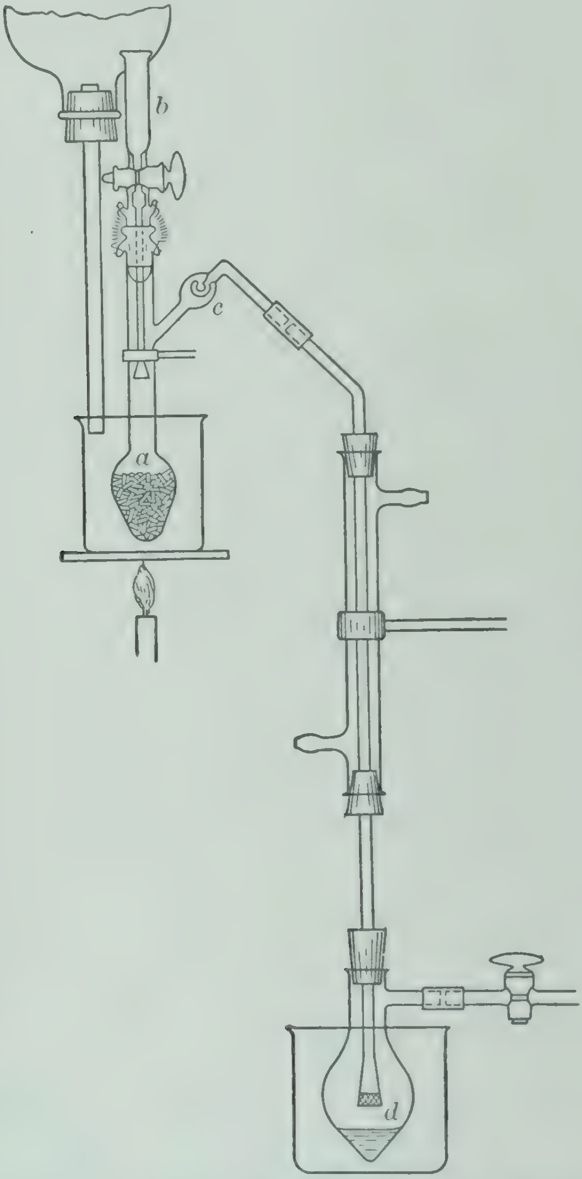


Fig. 61. Acetyl Apparatus. Explanation in Text.

Charging of the Distilling Flask. The sample is weighed in the weighing tube and transferred to the bottom of the distilling flask, which is then filled about three-fourths full with short pieces (4 mm.) of Pyrex glass rods. The ground-glass joint of the funnel is moistened with distilled water, inserted in the distilling flask, and fastened with short wire springs. Two milliliters of sulfonic acid solution is added through the funnel, and the stopcock is sealed with several drops of distilled water which is introduced through the funnel. When analyzing compounds containing halogen, 2 to 3 mg. of silver sulfate is added. The side arm of the flask is attached to the condenser with the freshly rinsed short rubber tubing, thereby effecting a glass-to-glass connection. The receiver is charged with 5 ml. of 0.01 *N* iodine solution to which about 1 gram of powdered potassium iodide had been added, and then it is attached to the rubber stopper on the lower end of the condenser; the height of the flask is adjusted so that the sintered-glass plate is approximately 1 cm. above the surface of the liquid. The side arm of the receiver is capped with a well-washed rubber nipple. The receiver is cooled by immersing it up to the side arm in a beaker of finely cracked ice, which is renewed from time to time during the hydrolysis. Finally, the distilling flask is tapped repeatedly to mix the sample intimately with the acid.

Heating. An asbestos board is placed as a shield between the burner and the condenser. The mixture is heated by bringing the water in the surrounding beaker to boiling. The level of water in the beaker is maintained during the period of heating by using an inverted bottle containing water, held well above the apparatus, and having a 1-cm. tube reaching just to the desired level in the beaker. During the course of the heating the flask is tapped vigorously at intervals in order to agitate its contents. Hydrolysis is continued for one hour for *O*-acetyl and approximately three hours for *N*-acetyl compounds. At the end of the hydrolysis the hot water is siphoned out of the beaker and replaced by ice-cold water. After cooling the apparatus for about five minutes, an aspirator is connected through a manometer and stopcock to the side arm of the receiver and the system evacuated to a pressure of 50 to 60 mm. The receiver is lifted, together with its cooling beaker, until the sintered plate is about 2 mm. from the bottom of the receiver and the water surrounding the distilling flask is heated. At first a few small bubbles will pass through the iodine solution, but the bubbling stops as soon as the pressure is equalized throughout the system. When the liquid in the distilling flask has completely distilled over, aided by occasional tapping, 1.5 to 2 ml. of water is run in through the funnel without breaking the vacuum or interrupting the heating. After the distilling flask has become dry, a second and finally a third portion of water are introduced in a similar manner. Heating is then continued until the distilling flask is completely dry and for about ten minutes thereafter. The receiver is then lowered with a gentle rotatory motion, until the sintered plate is 2 cm. above the solution. Next, the stopcock between the receiver and the manometer is closed and the connection between the stopcock and manometer is broken; by slowly opening the stopcock, the system is brought back to atmospheric pressure, after which the burner is extinguished.

Transfer of the Distillate and Titrations. The condenser is disconnected from the flask and rinsed three times with a small amount of distilled water. Drainage through the sintered-glass plate may be accelerated by application of slight suction through the side arm of the receiver. The outside of the lower part of the condenser is also washed with a small amount of distilled water, but the total volume of solution in the receiver should not exceed 20 ml. Then the iodine solution in the receiver is titrated with 0.01 N sodium thiosulfate solution in the usual manner (*first titration*). After completion of this titration, 2 ml. of the potassium iodate solution is added to the solution in the receiver, which is immediately stoppered with an Ascarite tube and placed in a beaker of water at about 35° C., in which it is allowed to stand for twenty minutes. Then the solution is titrated once more with the 0.01 N sodium thiosulfate solution as before (*second titration*).

Correction for Sulfur Dioxide. A small amount of sulfur dioxide might occasionally be formed as an interfering by-product in the hydrolysis. To correct for this, the receiver is originally charged with 5 ml. of 0.01 N iodine solution. This solution causes the conversion of the sulfur dioxide, or sulfurous acid, into sulfuric acid and hydriodic acid as follows:

$$H_2SO_3 + I_2 + H_2O = H_2SO_4 + 2HI$$

As a control, 5 ml. of the same 0.01 N iodine solution, to which 1 gram of potassium iodide and a few drops of acetic acid have been added, is titrated separately with the 0.01 N sodium thiosulfate solution (*third titration*). When measuring the 0.01 N iodine solution it is necessary to wait a few minutes before reading the meniscus. Usually, there ought to be no difference between the first and the third titration; differences up to 0.05 ml. are permissible, but if the difference is larger, the analysis is rejected. The difference, multiplied by 2, to account for the dibasicity of the sulfuric acid, is deducted from the second or main titration.

Calculation:

Log of net ml. 0.01 N sodium thiosulfate solution (second titration),
Plus log of factor (63370),
Plus negative log of weight of substance;
Antilog of total = percentage acetyl.

Remarks

The alkalimetric *O*- and *N*-acyl determination presented in this book is the method of H. Kuhn and H. Roth,[10] while the iodometric *O*- and *N*-acetyl determination given here is the method of A. Elek and R. A. Harte.[4]

Since the appearance of the micromethod of F. Pregl and A. Soltys,[16–18] who used *p*-toluene sulfonic acid as hydrolyzing agent, there have been two significant attempts to modify this determination in order to overcome certain inherent defects. The first of these is the method of A. Friedrich and co-workers,[7, 8] who simplified the apparatus and the manipulation. The use of a Claisen flask as hydrolyzing vessel and the omission of the U-tube make the apparatus simpler, and the replacement of the alkalimetric titration by the more efficient iodometric method is a desirable improvement. The regulation of the current of air passing through the apparatus during the distillation in vacuum to a rate of only 1 bubble in one to two seconds is rather difficult to maintain in practice, especially during the successive changes in temperature in the system.

The method of A. J. Bailey and R. J. Robinson [1] requires the refluxing of the sample for twelve to thirty-five hours in 0.04 N sodium hydroxide solution and titrating the excess alkali with 0.01 N hydrochloric acid. This method is applicable to substances which do not possess acidic functions either *per se*, such as lactones, lactams, etc.[15]

In addition to sodium hydroxide,[10, 23] barium hydroxide [9] and phosphotungstic acid [21] have been employed as hydrolyzing agents. The acid formed may be determined not only alkalimetrically [10, 16–19] or iodometrically [4, 7, 8, 18, 22] but also potentiometrically [16–19, 23] and by titration with ethanolamine.[21]

Methods involving transesterification in alcoholic solution of the acidic hydrolysis product have been described by K. Freudenberg and co-workers,[6] and others.[13]

An alkalimetric semi-micro method using alcoholic potassium hydroxide solution as the hydrolyzing agent has been described by E. P. Clark.[3] A comprehensive review of the literature on macro acetyl determination methods involving such hydrolyzing agents as sodium, potassium, calcium, barium, and ammonium hydroxides, as well as sulfuric, hydrochloric, and hydriodic acid, has been given by H. Meyer.[14] Successful extension of the methods for the determination of *O*- and *N*-acyl groups to include hydroxyl and primary and secondary amino groups by applying exhaustive acetylation of the alcohol or the amine in pyridine, and back titration of the excess acetylating agent, has been reported by a number of investigators.[2, 5, 20]

LITERATURE

1. Bailey, A. J., and Robinson, R. J., *Mikrochemie*, **15**, 233 (1934).
2. Cassidy, H. G., *Ind. Eng. Chem., Anal. Ed.*, **10**, 456 (1938).
3. Clark, E. P., *Ind. Eng. Chem., Anal. Ed.*, **8**, 487 (1936); **9**, 539 (1937).
4. Elek, A., and Harte, R. A., *Ind. Eng. Chem., Anal. Ed.*, **8**, 267 (1937).
5. Freed, M., and Wynne, A. M., *Ind. Eng. Chem., Anal. Ed.*, **8**, 278 (1936).
6. Freudenberg, K., and co-workers, *Ann.*, **433**, 230 (1923); **494**, 68 (1932); *Z. angew. Chem.*, **38**, 280 (1925).
7. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Vienna and Leipzig, 1933, pp. 160–170.
8. Friedrich, A., and co-workers, *Biochem. Z.*, **251**, 432 (1932); **286**, 20 (1936).
9. Kögl, F., and Postowsky, J. J., *Ann.*, **440**, 34 (1924).
10. Kuhn, R., and Roth, H., *Ber.*, **66**, 1274 (1933).
11. Leipert, T., *Mikrochemie, Pregl Festschrift*, 1929, p. 266.
12. Mayr, C., and Kerschbaum, E., *Z. anal. Chem.*, **73**, 321 (1928).
13. Merz, K. W., and Krebs, K. G., *Ber.*, **71B**, 302 (1938).
14. Meyer, H., "Analyse und Konstitutionsermittlung organischer Verbindungen," J. Springer, Berlin, 1903, pp. 332–343.
15. Pirie, N. W., *Biochem. J.*, **30**, 369 (1936).
16. Pregl, F., "Die quantitative organische Mikroanalyse," Third Edition, J. Springer, Berlin, 1930, pp. 216–222.
17. Pregl, F., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, pp. 235–245.
18. Pregl, F., and Soltys, A., *Mikrochemie*, **7**, 1 (1929).
19. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 191–200.
20. Stodola, F. H., *Mikrochemie*, **21**, 180 (1937).
21. Viditz, F. von, *Mikrochim. Acta*, **1**, 326 (1937).
22. Vieböck, F., and Brecher, C., *Ber.*, **63**, 3207 (1930).
23. Wolfrom, M. L., and co-workers, *J. Am. Chem. Soc.*, **58**, 490 (1936).

III. DETERMINATION OF GROUPS REACTIVE TO GRIGNARD REAGENT

Principle

The method is based upon quantitative interaction in a closed system of substances containing groups reactive to Grignard reagent with or without the evolution of gaseous hydrocarbons. Thus substances belonging to any of the types of compounds listed below are treated with a known amount of methyl magnesium iodide to yield methane quantitatively. The volume of methane formed is then measured in a suitably constructed methanometer.

Substances yielding one or more moles of methane:

Alcohols:	$ROH + CH_3MgI \rightarrow CH_4$
Thioalcohols:	$RSH + CH_3MgI \rightarrow CH_4$ [15b]
Phenols:	$ArOH + CH_3MgI \rightarrow CH_4$
Thiophenols:	$ArSH + CH_3MgI \rightarrow CH_4$
Acids:	$RCOOH + CH_3MgI \rightarrow CH_4$
	$RSO_2OH + CH_3MgI \rightarrow CH_4$
Amides:	$RCONH_2 + 2CH_3MgI \rightarrow 2CH_4$ [15b]
	$RCONHR + CH_3MgI \rightarrow CH_4$ [15b]
Sulfonamides:	$RSO_2NH_2 + 2CH_3MgI \rightarrow 2CH_4$
	$RSO_2NHR + CH_3MgI \rightarrow CH_4$
Amines:	$RNH_2 + 2CH_3MgI \rightarrow 2CH_4$
	$R_2NH + CH_3MgI \rightarrow CH_4$
Nitro compounds:	$RNO_2 + CH_3MgI \rightarrow CH_4$ [7]
Water:	$H_2O + 2CH_3MgI \rightarrow 2CH_4$ [3,15b]

After the evolution of methane due to active hydrogen, the unused Grignard reagent is reacted with a known quantitity of aniline and the volume of methane thus generated is again measured. Thus, by difference, such groups as react with methyl magnesium iodide *without* the evolution of methane can be determined by the amount of Grignard reagent consumed, as indicated below:

1 mole of Grignard reagent is consumed by:

Aldehydes:	$RCHO + CH_3MgI \longrightarrow RCHOMgI$
Ketones:	$R_2CO + CH_3MgI \longrightarrow R_2COMgI$
Halogen compounds:	$RCH_2X + CH_3MgI \longrightarrow RCH_2CH_3$
Nitriles:	$RCN + CH_3MgI \longrightarrow RC(CH_3){:}NMgI$
Isonitriles:	$RNC + CH_3MgI \longrightarrow RN{:}C(CH_3)MgI$

2 moles of Grignard reagent are consumed by:

Acids: $\mathrm{RCOOH} + 2\mathrm{CH_3MgI} \xrightarrow{-\mathrm{CH_4}} \mathrm{R(CH_3)C(OMgI)_2}$

Acid Halides: $\mathrm{RCOX} + 2\mathrm{CH_3MgI} \longrightarrow \mathrm{R(CH_3)_2COMgI}$

Esters: $\mathrm{RCOOR} + 2\mathrm{CH_3MgI} \longrightarrow \mathrm{R(CH_3)_2COMgI}$

Apparatus

The apparatus, which is shown in Fig. 62, consists of two parts: the methane generator and the methanometer. They are connected with a rubber tubing 16 cm. long having a bore of 3 mm. and an outer diameter of 9 mm.

Methane Generator. This part of the apparatus consists of the removable reaction flask (*a*), the two burets (b_1 and b_2), the Grignard reagent stock flask or reagent vessel (*c*), and two three-way stopcocks, one of which is on the bottom (*S–a*) and the other on the top (*S–b*). The Grignard reagent stock flask has a capacity of 50 ml. and is provided with a ground-glass stopper and two outlets. The outlet on the top leads to stopcock *S–b*. The outlet on the side is a capillary tubing one arm of which extends to the bottom of the Grignard reagent vessel; the other arm leads to buret b_2, where it forms an automatic zero-point adjustment. The reaction flask *a*, which has a capacity of 8 ml., is attached by means of a ground-glass joint to a hollow stopper, or head. The capillary inlet tube *d*, which may be connected with either buret b_1 or b_2, depending upon the position of stopcock *S–a*, is sealed to *S–a* and extends through the hollow stopper into the reaction flask. Another capillary tubing leads from the hollow stopper up to the right side arm of stopcock *S–b*. Buret b_2 is graduated to 0.02 ml., and has a capacity of 2 ml.; it serves for the measurement and the introduction of the Grignard reagent. Buret b_1 is also graduated to 0.02 ml. but has a capacity of 1 ml. and is used for the introduction of the aniline. An automatic shaking device is connected to the apparatus by means of a wire attached to the capillary inlet tube.

Methanometer. This consists of two parallel gas burets (b_3 and b_4), each having a capacity of 7 ml.; both are graduated to 0.02 ml. The methane buret b_4 carries two three-way stopcocks at its upper end, which facilitate the admittance of methane or dry nitrogen, respectively. Buret b_3 is open on the top to permit establishment of equilibrium with the atmospheric pressure. Its curved bottom is sealed to buret b_4 and is provided with an outlet which connects both burets by means of a rubber tubing to the mercury leveling bulb, which is suspended from a rack having a pinion adjustment.

Reagents

Nitrogen [11] should be of 99.9% purity, as used in the manufacture of electric-light bulbs; it is used for the preparation of the Grignard reagent as well as during the determination to provide an inert atmosphere. The nitrogen tank should be equipped with a reduction valve

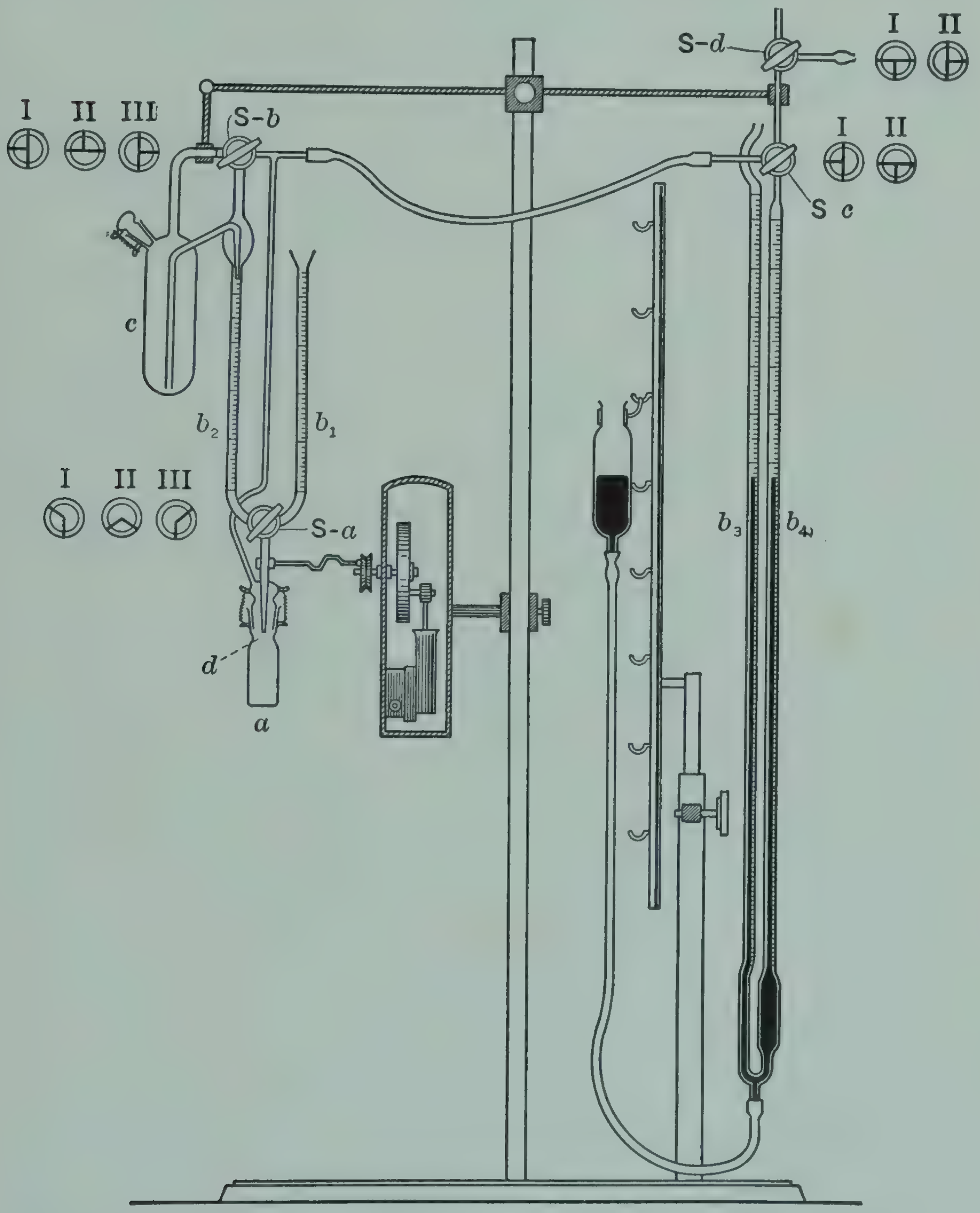

S-d
I
II
I
II
III
S-b
I
II
S c
c
b_2
b_1
I
II
III
S-a
b_3
b_4
d
a

Fig. 62. Apparatus for the Determination of Groups Reactive to Grignard Reagent. Explanation in Text.

for the regulation of the flow of nitrogen, which is led through a mercury valve bottle, then through a wash bottle containing concentrated sulfuric acid and a U-tube filled with Anhydrone. If it is necessary to purify the nitrogen and free it from oxygen, it is passed over metallic copper heated to about 700° C. and bubbled successively through 50% potassium hydroxide solution and concentrated sulfuric acid.

Solvents

Anethole [20] is a very good solvent which can readily be purified by distillation (m.p. 22°; b.p. 235°); it is stored in an absolutely dry, ground-glass stoppered reagent bottle over metallic potassium. The best solvent, however, is *pyridine*[1] (b.p. 113–115°). A mixture of pyridine and anethole (1 : 5) is most satisfactory. The pyridine is freed from its homologs by treatment with perchlorate followed by distillation in vacuum. To dry it completely it is placed in a strong, ground-glass-stoppered bottle containing some coarse, freshly ignited barium oxide and shaken for six hours. Then the pyridine is quickly filtered through a fluted filter paper into another ground-glass-stoppered reagent bottle which also contains some freshly ignited barium oxide. After standing for two to three days the pyridine is ready for use.

Amyl ether (b.p. 187–190°) is used as the solvent for the Grignard reagent. It is redistilled and is stored over metallic sodium in a ground-glass-stoppered bottle which has been dried at 100° C.

Grignard Reagent. In a 250-ml. round-bottomed flask are placed 0.6 gram magnesium, 2.5 ml. methyl iodide, and 50 ml. freshly distilled amyl ether. This flask is provided with a ground-glass condenser and a sealed-in inlet tube. The flask is charged through the inlet tube with nitrogen which has been dried by passing it through a wash bottle containing concentrated sulfuric acid and a U-tube filled with Anhydrone. Before the start of the reaction the flow of nitrogen is reduced to about 1 bubble per second. Water is passed through the condenser jacket and, after the reaction has been started by dropping a crystal of iodine into the flask, it is heated for two hours on the water bath. To remove the last traces of methyl iodide the condenser outlet is connected to a suction pump, and the rubber tubing connecting it to the pump is closed with a pinch clamp. The burner is extinguished and the water passing through the condenser jacket shut off, as is the flow of nitrogen. The pinch clamp regulating the vacuum from the suction pump is then slowly opened, causing the contents of the flask to boil which, with the aid of the suction, removes the methyl iodide completely, although a small amount of amyl ether is also lost. As soon as this pinch clamp is fully opened, the pinch clamp beyond the nitrogen inlet tube is opened to let 1 bubble of nitrogen per second pass through the wash bottle. After fifteen minutes the pinch clamp regulating the vacuum is closed and the one throttling the delivery of nitrogen slowly opened completely so that the flask is again filled with nitrogen. The rubber connections are then removed and the top of the condenser as well as the inlet tube are closed with tight-fitting rubber stoppers or rubber tubings. It is advisable to determine the strength of the reagent before transferring it to the apparatus by dissolving about 5 ml. of the reagent in an excess of 0.1 N hydrochloric acid and titrating it back with 0.1 N sodium hydroxide. The Grignard reagent should be of 0.4 to 0.5 molar concentration.

Filling the Apparatus with the Grignard Reagent

The apparatus is thoroughly cleaned with dilute hydrochloric acid, water, alcohol, and acetone, and then dried. All grease must be removed from the stopcocks. Stopcocks *S–b*, *S–c*, and *S–d* are greased to transparency, but not excessively, with a vaseline-lanolin mixture. Stopcock *S–a* is lubricated with special stopcock grease, consisting of a mixture of cylinder oil and graphite (1 : 6), but no trace of the lubricant is allowed to enter the bore. The two sections of the apparatus are attached to each other with a good pressure tubing, and the gas burets b_3 and b_4 are connected to the drying apparatus and valve bottle. Stopcocks *S–a*, *S–b*, *S–c*, and *S–d* are turned to position I, shown in the diagram, and the reaction flask, which is greased sparingly on its upper rim, is attached. Nitrogen is passed through the apparatus for ten minutes at a rate of about 1 bubble per second, and, while nitrogen is still passing through, the Grignard reagent is poured in through the opening of vessel (*c*) with a funnel having a bent stem and a plug of glass wool, in such a manner that the funnel does not run empty until the vessel is filled within 1 cm. of the inlet. A white layer forms on the surface of the reagent, due to the moisture and oxygen absorbed from the air, which, however, is retained by the glass wool. The ground-glass joint of the reagent vessel is also greased sparingly on the upper rim with the vaseline-lanolin mixture. At this stage the Grignard reagent usually shows a slight turbidity which clears up after several hours. Finally, stopcock *S–a* is turned to position III.

Determination of the Blank

Stopcocks *S–b*, *S–c*, and *S–d* are turned to position I and *S–a* to III. With a dry pipet, 0.5 ml. of anethole is introduced into the dry and still warm reaction flask. The upper rim of the flask is greased with the special stopcock grease and the flask attached to the apparatus. It is placed in a water bath of room temperature, and nitrogen is passed through the apparatus for eight minutes. Buret b_2 is filled with the Grignard reagent by raising the mercury bulb and turning stopcocks *S–a* and *S–b* to position II; the buret is filled when the maximum pressure is reached in the reagent vessel, e.g., when nitrogen is escaping from the mercury valve bottle. Stopcock *S–b* and then *S–a* are turned counterclockwise to position III, utilizing the excess pressure to force the reagent into the buret. As soon as the reagent rises beyond the zero point adjustment stopcock *S–b* is turned to position I, causing the excess to flow back into the reagent vessel. The excess pressure may not be sufficient to force the reagent into the buret if the reagent vessel is completely filled, in which case stopcocks *S–c* and *S–a* are turned to position II and the buret filled by lowering the mercury bulb. The excess is again siphoned back into the reagent vessel by raising the mercury bulb. The reagent vessel and buret are shut off from the rest of the apparatus by turning stopcock *S–b* to position I. Stopcock *S–c* is turned to I and *S–a* to III to permit nitrogen to pass through the apparatus for the purpose of temperature equalization. After ten minutes the mercury in gas burets b_3 and b_4 is raised to zero and stopcock *S–c* and then *S–a* are turned to position II. The mercury bulb is lowered approximately 10 cm. and stopcock *S–a* is turned clockwise to position I; 0.5 ml. of Grignard reagent is run from the Grignard buret (b_2) into the reaction flask, and stopcock *S–a* is turned back to position II. After the reaction flask has been shaken for five minutes, the mercury buret b_3 is raised to zero and burets b_2 and b_4 are read. The difference between b_4 and b_2 represents the blank volume (v_b).

Next, the reaction flask is immersed in boiling water and shaken for ten minutes. To facilitate the release of the gases during this procedure, the mercury bulb is lowered 3 to 4 cm. to create a slight underpressure. The flask is then cooled for ten minutes in water at room temperature, with shaking. The mercury in buret b_3 is raised to zero and b_4 is read again. The blank should show no increase after heating the reagent, except when pyridine is used. This substance does give a higher blank when heated, but since it is also necessary to heat the reaction flask when an unknown substance is analyzed, the blank must be carried out under the same conditions; the blank volume for 0.5 ml. of Grignard reagent should not be higher than 0.2 ml.[13, 18, 20]

To determine the molarity of the reagent, buret b_1 is filled with aniline and the volume is read accurately. The mercury bulb is lowered about 10 cm.; stopcock *S–a* is turned to position III and 0.6 to 0.9 ml. of aniline is run in, and *S–a* is turned to position II and the reaction flask is shaken for five minutes. The mercury in buret b_3 is then raised to zero and b_4 is read. The amount of reagent and aniline used is deducted from the gas volume in the buret to determine the volume of methane produced by the reagent (v_a). Its strength is expressed in milliliters of methane at 0° C. and 760 mm. pressure per milliliter of reagent, or by its molarity. It is necessary to determine the strength of the reagent only when working with substances which react with the Grignard reagent but do not liberate methane. During the various manipulations of the apparatus it is advisable to touch the gas burets and glass tubings as little as possible.

Procedure

Preparation of the Apparatus. The reaction flask is removed from the apparatus and cleaned with ethyl alcohol and dilute hydrochloric acid; the grease is removed from its neck with cotton wound around a knurled iron wire and moistened with acetone. It is finally rinsed with acetone and dried at 100° C. The ground-glass joint of the reaction flask is also thoroughly cleaned with cotton moistened with acetone. Stopcock *S–a* is then turned to position III, and some alcohol, and then some acetone, are introduced through buret b_1. Should there be deposits of magnesium salts in the capillary, they are removed with dilute hydrochloric acid. While the reaction flask and the pipet are permitted to dry in a drying block, a slow stream of nitrogen is passed through the apparatus, with stopcocks *S–d* and *S–c* in position I; air is drawn through capillary *d* at the same time to dry buret b_1, and after five minutes the apparatus is ready for the determination.

Introduction of the Sample. Enough substance is weighed in the weighing tube to yield about 1 ml. of methane and is transferred to the still warm reaction flask. With the warm pipet 0.5 ml. of anethole, or pyridine, or a mixture of both, is added; then the flask is attached, stopcocks *S–d, S–c, S–b* are turned to position I, *S–a* is turned to position III, and nitrogen is passed through the apparatus. Then the reaction flask is immersed in water at room temperature and shaken to bring about equalization of temperature.

If the substance does not dissolve during the shaking, the reaction flask is heated to 100° C. on the heating block. The substance must not precipitate out upon cooling.

Generation of Methane. After ten minutes, when the temperature has become equalized, buret b_2 is filled with the reagent. The mercury in gas burets b_3 and b_4 is raised to zero and stopcocks *S–c* and *S–a* are turned to position II. The mercury bulb is lowered about 10 cm., stopcock *S–a* is turned clockwise to position I, and about 0.5 ml. of reagent, or an excess over the theoretical amount, is run into the reaction flask. Then stopcock S–a is turned to position II. The reaction flask is shaken at room temperature for five minutes, after which the mercury in buret b_3 is raised to zero and burets b_2 and b_4 are read. The blank volume as well as the amount of reagent added are deducted to obtain the volume of methane formed by the substance. The mercury bulb is lowered again, the reaction flask is then immersed in boiling water, and the shaking is continued for ten minutes. The hot water is then replaced by water at room temperature and the reaction flask shaken for another ten minutes. The mercury in gas buret b_3 is raised to zero and buret b_4 is read.

Addition of Aniline. After completion of the reaction and readings buret b_1 is filled with aniline and read accurately. About 0.7 ml. of aniline is run into the reaction flask by lowering the mercury bulb and turning stopcock S–a to position III. Stopcock S–a is turned to position II and the reaction flask is shaken for five minutes at room temperature. The mercury in buret b_3 is raised to zero and buret b_4 is read. The volume of aniline added is then deducted from the increased gas volume and the blank added to obtain the volume of methane corresponding to the amount of unused reagent. The time required for the entire analysis, including the weighing of the substance, is about one hour.

Calculation:

Three calculations are involved (see p. 298):

1. The calculation of the volume of methane produced by the sample at room temperature (v_t).
2. The calculation of the volume of methane produced by the sample at elevated temperature (v_T).
3. The calculation of the final volume of methane (v_f).

Remarks

The method for the determination of groups reactive to Grignard reagent as presented in this manual is the method of A. Soltys.[20] This method, which is based upon the observations of L. Tschugaeff,[21] the macromethods of T. Zerewitinoff [22] and E. P. Kohler and co-workers,[8] and the micromethod of B. Flaschenträger,[4] appears to incorporate all the latest improvements. Specifically, it has the advantage that, in addition to the determination of active hydrogen, it allows the quantitative determination of any other atomic or molecular group or groupings capable of reacting with a Grignard reagent.[2, 5, 7–9, 14, 15a–17, 19]

Gas volumetric methods for the determination of active hydrogen alone, which consequently involve a simpler apparatus, have been devised by H. Roth,[15a, 17] W. Fuchs and co-workers,[5] and others.[4] The methane evolved may also be determined manometrically [10] as well as gravimetrically in the form of carbon dioxide and water. as demonstrated by R. N. Evans and co-workers.[3]

Complete solubility of the substance in the solution of the Grignard reagent is absolutely necessary. As solvents, aliphatic (di-*n*-butyl, di-*n*- and isoamyl, etc.) and aromatic ethers (anisole,[6] anethole,[20] etc., as well as aromatic hydrocarbons (xylene,[19] etc.) and pyridine,[1, 16, 20] have been suggested. The solvents must be perfectly dry, a condition which is not always easy to fulfill. It was found that even metallic sodium is not sufficient, and the use of phosphorus pentoxide in cases of non-reactive solvents had to be resorted to. The sensitivity of the Grignard reagent towards moisture [6] has been repeatedly employed for the determination of water of crystallization.[15b] R. N. Evans and co-workers [3] utilized this property of Grignard reagents in their gravimetric method for the determination of moisture in insulating materials. Absence of oxygen in the apparatus is highly desirable,[6, 12] and therefore the use of nitrogen [11] in all methods involving Grignard reagents is preferred.

LITERATURE

1. Arndt., F., and Nachtwey, P., *Ber.*, **59**, 448 (1926).
2. Clutterbuck, P. W., and co-workers, *J. Biochem.*, **29**, 300 (1935).
3. Evans, R. N., Davenport, J. E., and Revukas, A. J., *Ind. Eng. Chem., Anal. Ed.*, **12**, 301 (1940).
4. Flaschenträger, B., *Z. physiol. Chem.*, **146**, 219 (1923).
5. Fuchs, W., Ishler, N. H., and Sandhoff, A. G., *Ind. Eng. Chem., Anal. Ed.*, **12**, 507 (1940).
6. Hibbert, H., and Sudborough, J. J., *J. Chem. Soc., Proceedings*, **19**, 285 (1903).
7. Houben, J., and Weyl, H., "Die Methoden der organischen Chemie," G. Thieme, Leipzig, 1924, Vol. IV, p. 201.
8. Kohler, E. P., Stone, J. F., and Fuson, R. C., *J. Am. Chem. Soc.*, **49**, 3181 (1927).
9. Krellwitz, L., Dissertation, University of Freiburg, 1914.
10. Lüttgens, W., and Negelein, E., *Biochem. Z.*, **269**, 177 (1934).
11. Marrian, P. M. and G. F., *Biochem. J.*, **24**, 276 (1930).
12. Meisenheimer, J., and Schlichenmayer, *Ber.*, **61**, 2029 (1928).
13. Neuworth, M., Carnegie Inst. Technol., Coal Research Lab., Pittsburgh, Pa., private communication.
14. Oddo, B., *Ber.*, **44**, 2048 (1911).
15. Pregl, F., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, (*a*) pp. 192–204; (*b*) p. 194.
16. Roth, H., *Mikrochemie*, **11**, 140 (1932).
17. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, pp. 156–166.

18. Schmitt, R. B., Loyola College, Baltimore, Md., private communication.
19. Shtuber E. Y., and Dobromyslova, A. V., *J. Applied Chem.*, (*U. S. S. R.*), **11**, 704 (1938).
20. Soltys, A., *Mikrochemie*, **20**, 107 (1936).
21. Tschugaeff, L., *Ber.*, **35**, 3912 (1902).
22. Zerewitinoff, T., *Ber.*, **40**, 2033 (1907); **41**, 2233 (1908); **42**, 4802 (1909); **43**, 3590 (1910); **47**, 1659, 2417 (1914).

IV. OTHER METHODS

In addition to the more general structural analytical methods given in the preceding chapters, a number of special methods suitable for more or less specific substances or conditions have been developed.

Of these the method of D. D. Van Slyke [49] for the determination of *primary amines* is one of the most widely used.[32, 39a, 41a, 42] Primary amines are treated in a closed system with nitrous acid to yield nitrogen, i.e.:

$$RNH_2 + NaNO_2 + CH_3COOH \rightarrow H_2O + CH_3COONa + N_2$$

The nitrogen is determined *volumetrically.* The apparatus employed is somewhat similar to the one described in the manometric determination of carbon (p. 140); it consists essentially of the reaction chamber with the graduated dropping funnel, the gas buret, and the Hempel pipet. The solution containing the primary amine is introduced through the graduated dropping funnel into the reaction chamber, which has previously been filled with sodium nitrite solution and acetic acid. The reaction is usually finished in three to five minutes. After purification of the reaction gases by means of the alkaline permanganate solution contained in the Hempel pipet, the volume of the residual nitrogen is determined. A blank is necessary.

Electrometric and photoelectric methods for the quantitative determination of certain types of organic compounds have come more and more into use. The instruments employed in such procedures have been extensively reviewed by R. H. Müller.[35, 35a] Conductometric (salts of organic acids),[4] potentiometric (reducing sugars),[37] vitamins,[25] photoelectric (hydroxy acids,[35a, 52] vitamins [9, 13, 43]), as well as polarographic methods [19, 26, 36] have been found applicable.

A number of catalytic *micro hydrogenation* methods for the determination of double bonds in organic compounds have been devised.[3, 12, 21, 28, 51] Platinic oxide and palladium oxides,[1] or platinum black,[27] are used as catalysts. The amount of hydrogen consumed may be determined *volumetrically* [38, 45, 46] or *manometrically*.[20, 24, 39d, 41d, 48, 50, 53]

$$R_2C{=}CR_2 \xrightarrow{+H_2} R_2CH \cdot CHR_2$$

In a rather simple all-glass distillation apparatus *isopropylidene groups* may be determined *iodometrically* as acetone.[2, 5, 7, 8, 11, 14–18, 22, [23, 29a, 30, 31, 33, 34, 39c, 40, 41c, 47] The acetone is formed either by hydrolysis with 1 *N* sulfuric acid (*O*-isopropylidene groups, acetone sugars, etc.), or by ozonalysis (*C*-isopropylidene groups, unsaturated compounds, etc.). After destruction of interfering by-products by oxidation with 1 *N* potassium permanganate solution, the acetone is converted quantitatively into iodoform by using 2 *N* sodium hydroxide solution and an excess, but known quantity, of 0.05 *N* iodine solution as follows:

$$(CH_3)_2C{=} \rightarrow (CH_3)_2C{=}O$$

$$CH_3COCH_3 + 3I_2 + 4KOH \rightarrow CHI_3 + CH_3COOK + 3KI + 3H_2O$$

The excess iodine is then titrated with 0.05 *N* sodium thiosulfate solution in the usual manner.

By oxidation with chromic-sulfuric acid mixture, and using an apparatus similar to the one employed in the determination of acyl groups (p. 252), *terminal methyl groups* in a number of substances can be oxidized to acetic acid, which is then determined *alkalimetrically*.[30a, 33, 39b, 41b, 44]

The *optical rotation* of small amounts of optically active substances can be determined by using a micropolarimeter tube and an ordinary polarimeter.[6, 10, 39e, 41e] Such a micropolarimeter tube is constructed like the conventional tubes, except that the tube itself is a thick opaque glass tube 5 cm. long, 1.4 cm. in outer and 2.5 mm. in inner diameter. The cover glass and the caps are the same as in an ordinary polarimeter tube. The solution is prepared by weighing the substance in the weighing tube and placing it into a suitable stoppered weighing bottle, which is then weighed with the substance and again after the addition of the solvent.

Calculation:

$$[\alpha]_D^t = \frac{100\alpha}{lc}$$

α = observed rotation;
c = concentration of solution (grams of substance in 100 ml. solvent);
l = length of tube in decimeters.

LITERATURE

1. Adams, R., and Shriner, L. R., *J. Am. Chem. Soc.*, **45**, 107 (1923); **46**, 1683 (1924).
2. Bell, D. J., and Harrison, K., *J. Chem. Soc.*, **1939**, 350.

3. Bretschneider, H., and Burger, G., *Chem. Fabrik*, **10**, 124 (1937).
4. Dittmer, K. H., and Gustavson, R. G., *Ind. Eng. Chem., Anal. Ed.*, **12**, 297 (1940).
5. Doeuvre, J., *Bull. soc. chim.*, (4) **39**, 1594 (1926).
6. Donau, J., *Monatsh.*, **29**, 333 (1908).
7. Elsner, H., *Ber.*, **61**, 2364 (1928).
8. Escourrou, R., *Bull. soc. chim.*, (4) **43**, 1088 (1928).
9. Ewing, D. T., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **12**, 297 (1940).
10. Fischer, E., *Ber.*, **54**, 1979 (1921).
11. Fischer, F. G., and Löwenberg, K., *Ann.*, **494**, 263 (1932).
12. Foresti, B., *Ann. chim. applicata*, **26**, 207 (1936).
13. French, R. B., *Ind. Eng. Chem., Anal. Ed.*, **12**, 351 (1940).
14. Freudenberg, K., and co-workers, *Ber.*, **61**, 1735 (1928).
15. Goodwin, L. F., *J. Am. Chem. Soc.*, **42**, 39 (1919).
16. Grignard, V., and co-workers, *Compt. rend.*, **177**, 669 (1923); **187**, 270, 330 (1928); *Bull. soc. chim.*, (4) **35**, 932 (1924); **45**, 809 (1929).
17. Grün, A., *Ber.*, **59**, 695 (1926); **62**, 473 (1929).
18. Harries, C., *Ann.*, **343**, 311 (1906); **374**, 288 (1910); **410**, 8 (1915).
19. Hohn, H., "Chemische Analyse mit dem Polarographen," J. Springer, Berlin, 1937.
20. Hyde, J. F., and Sherp, H. W., *J. Am. Chem. Soc.*, **52**, 3359 (1930).
21. Jackson, H., and Jones, R. N., *J. Chem. Soc.*, **1936**, 895.
22. Judefind, W. L., and Reid, E. E., *J. Am. Chem. Soc.*, **42**, 1043 (1920).
23. Karrer, P., and co-workers, *Helv. Chim. Acta*, **14**, 435 (1931).
24. Kautzky, H., and Baumeister, W., *Ber.*, **64**, 2446 (1931).
25. Kirk, M. M., and Tressler, D. K., *Ind. Eng. Chem., Anal. Ed.*, **11**, 322 (1939).
26. Kolthoff, I. M., and Lingane, J. J., *Chem. Rev.*, **24**, 1 (1939).
27. Köppen, R., *Z. Elektrochem.*, **38**, 938 (1932).
28. Kuhn, R., and Möller, E. F., *Z. angew. Chem.*, **17**, 145 (1934).
29. Kuhn, R., and Roth, H., *Ber.*, (*a*) **65**, 1285 (1932); (*b*) **66**, 1274 (1933).
30. Kuhn, R., and co-workers, *Ber.*, **65**, 651 (1932); (*a*) *Z. angew. Chem.*, **44**, 847 (1931); *Helv. Chim. Acta*, **12**, 64 (1929).
31. Lax, H., *Biochem. Z.*, **125**, 262 (1921).
32. Matukawa, D., *Chem. Abs.*, **34**, 2731 (1940).
33. Messinger, J., *Ber.*, **21**, 2910, 3366 (1888); **23**, 2756 (1890).
34. Moses, C. G., and Reid, E. E., *J. Am. Chem. Soc.*, **54**, 2101 (1932).
35. Müller, R. H., *Ind. Eng. Chem., Anal. Ed.*, **12**, 57 (1940) (a review of electrical instruments); (*a*) **11**, 1, (1939).
36. Müller, O. H., *J. Chem. Education*, **18**, 65, 111, 172, 227 (1941).
37. Niederl, J. B., and Müller, R. H., *J. Am. Chem. Soc.*, **51**, 1356 (1929).
38. Prater, A. N., and Haagen-Smit, A. J., *Ind. Eng. Chem., Anal. Ed.*, **12**, 705 (1940).
39. Pregl, F., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935, (*a*) p. 204; (*b*) p. 246; (*c*) p. 249; (*d*) p. 259; (*e*) p. 301.
40. Richter-Quittner, M., *Biochem. Z.*, **93**, 163 (1919).
41. Roth, H., and Daw, E. B., "Quantitative Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937, (*a*) p. 166; (*b*) p. 201; (*c*) p. 204; (*d*) p. 212; (*e*) p. 243.
42. Rudy, H., and Page, I. H., *Z. physiol. Chem.*, **193**, 251 (1930).

43. Schumacher, A. E., and Heuser, G. F., *Ind. Eng. Chem., Anal. Ed.*, **12**, 203 (1940).
44. Simon, L. J., *Compt. rend.*, **170**, 514 (1920); **174**, 1706 (1922); **175**, 167, 525, 768, 1070 (1922); **176**, 1065 (1923); **177**, 265 (1923).
45. Slotta, K. H., and Blanke, E., *J. prakt. Chem.*, **143**, 3 (1935).
46. Smith, J. C., *J. Biol. Chem.*, **96**, 35 (1932).
47. Svanberg, O., and Sjöberg, K., *Ber.*, **56**, 1452 (1923).
48. Tsuda, K., and Sakamoto, S., *J. Pharm. Soc. Japan*, **57**, 1037 (1937).
49. Van Slyke, D. D., *J. Biol. Chem.*, **9**, 195 (1911); **12**, 275 (1912); **16**, 121 (1913); **23**, 407 (1915).
50. Warburg, O., and Christian, W., *Biochem. Z.*, **266**, 387 (1933).
51. Weygand, C., and Werner, A., *J. prakt. Chem.*, **149**, 330 (1937).
52. Williams, A. S., Müller, R. H., and Niederl, J. B., *Mikrochemie*, **9**, 269 (1931).
53. Willstaedt, H., *Ber.*, **68**, 333 (1935).

APPENDIX

I. THE TEACHING OF QUANTITATIVE ORGANIC ELEMENTARY MICROANALYSIS

The methods described in this book can be adapted wholly or in part to form a course in quantitative organic elementary microanalysis. Depending upon the requirements and facilities available, it may be patterned after the course given at New York University, Washington Square College,[19] which is described in this chapter. At New York University it is a graduate course and only students possessing at least a B.Sc. degree, or its equivalent, are accepted. These students have already finished their preliminary studies and possess a fairly uniform average chemical education. Quantitative inorganic analysis is made a prerequisite; no special consideration is given to whether or not the student is majoring in organic or any other branch of chemistry, as it has been found that the training is beneficial for students of any of the experimental sciences, whether physics, biology, or chemistry, because they are made to realize the importance of exactness, cleanliness, and the painstaking following of experimental procedures. Deviations from these requirements certainly will prove fatal to the obtaining of acceptable results, and hence the student gradually acquires what F. Pregl called "chemical asepsis."

The course is given for one term, over a period of fourteen to fifteen weeks; each student is required to reserve one full day a week for the course, and a group of four students comprises the day's class, which makes individual instruction and full supervision readily possible. Each student is assigned his own balance and other apparatus, thereby avoiding interference from other individuals. The balances are alternated; that is, during one period the student uses a microanalytical balance and during the next period an ordinary analytical balance, so that every student is trained in handling and using both types of balances.

The various apparatus, which are permanent set-ups and available in duplicate so that two students can always carry out the same determination independently without interfering with each other, are arranged on tables adjacent to the walls of the room, with the exception of the two Dumas apparatus, which are on a center table. The equipment furnished each student is simple: it consists of a microspatula, a small camel's-hair brush, a pair each of steel and ivory-tipped forceps, a knurled iron wire, and a wire fork, all of which are kept in a large test tube. In addition, each student is provided with a microdesiccator, which carries the same number as the balance to avoid confusion, a platinum boat, a weighing tube, and a set of weights. The counterpoises for the platinum boat and weighing tube are kept in a small wooden box labeled with the student's name.

Working Schedule. There is a definite assignment of work for every day or period which has to be completed, although an additional day is set aside at the end of the course to permit the student either to make up for lost time or to repeat some of the determinations with which he had the most difficulty. The table below illustrates a typical working schedule for the duration of the term:

Total number of periods (days)	15
	Periods
Weighing: Determination of sensitivity and precision; calibration of weights.	
Microanalytical balance (pp. 15-21, 26-30)	1
Ordinary analytical balance (pp. 23-26, 26-30)	1
Determination of metals and neutralization equivalent (p. 67)	1
Determination of aminoid nitrogen (Kjeldahl) (pp. 72-74)	1
Gasometric determination of nitrogen (Dumas) (pp. 89-95)	3
Determination of carbon and hydrogen (pp. 123-126, 128-132)	4
*Determination of halogen (Carius) (pp. 156-160)	1
*Determination of sulfur (Carius) (pp. 185-188)	1
Determination of molecular weight (cryoscopic method) (p. 218)	1
Laboratory examination:	
Duplicate carbon and hydrogen analysis and duplicate analyses on same sample for one other element, or molecular-weight determination	1
Total	15

* Samples are weighed and pressure tubes are filled and sealed the preceding day.

The training period outlined above does not include the setting up of the more complicated apparatus, although the student is given an opportunity to learn how to fill the absorption tubes and the combustion tubes for the Dumas and the carbon and hydrogen apparatus. The student is instructed to perform first duplicate analyses of known substances and then at least one or more duplicate analyses of research compounds. The following minimum accuracy is required:

Kind of Determination	Allowable Error
Metals	±0.2%
Neutralization equivalent	±2.0%
Nitrogen (Kjeldahl)	±0.2%
Nitrogen (Dumas)	±0.2%
Carbon and hydrogen	±0.3%
Halogen	±0.3%
Sulfur	±0.3%
Molecular weight (cryoscopic)	±5.0%

The various methods are taught by having the instructor demonstrate the first determination of each procedure and then supervise closely while the student carries out the subsequent analyses, until the student has acquired the necessary experience to work independently. To teach the student to utilize his time to the fullest extent, he is compelled to observe strictly the time schedule outlined in the procedure; working by the stopwatch is resorted to in determinations where observance of exact time limits is of importance. At the end of the day the student is required to enter the results obtained in a notebook, which is handed to the instructor, who compares them with the theoretical values. The instructor also keeps a separate permanent record of every analysis made by the student, as well as a record of the sensitivity and precision of the balances before and after the course. For research substances separate reports, signed by the student, are made out which give all the important data pertaining to the analyses.

Examination unknowns are handed out in the last period of the course. They consist of the total analysis of one unknown substance, usually a research compound, requiring, in addition to quantitative carbon and hydrogen determination, at least one, but not more than two, further independent determinations, such as nitrogen, metal, neutralization equivalent, or molecular weight. If time permits, any one of the other standard quantitative microdeterminations, such as alkoxyl, may be taken up and practiced.

The course in quantitative organic microanalysis has been given at New York University in the systematic manner outlined above since its incorporation in the curriculum of the Graduate School with the following attendance:

Year	1929	1930	1931	1932	1933	1934	1935	1936	1937	1938	1939	1940
No. of Students	12	12	15	18	19	19	19	18	14	16	14	20
Total	196											

In the table below are shown the average number of the various determinations taught during the course in 1940, which consists of fourteen periods or days of eight hours each, and one period for the laboratory examination at the end of the term.

Kind of Analyses	Total Analyses	Known Substances	Research Substances
Metal	3	2	1
Neutralization equivalent	3	2	1
Nitrogen (Kjeldahl)	6	3	3
Nitrogen (Dumas)	12	4	8
Carbon and hydrogen	18	6	12
Halogen (Carius)	4	2	2
Sulfur (Carius)	3	2	1
Molecular weight	4	2	2
Total	53	23	30

During the period from 1934 to 1940 the following number of analyses of both known and research substances were carried out by the 120 students who have taken the course:

Kind of Analyses	Total Analyses	Known Substances	Research Substances
Metal	639	430	209
Neutralization equivalent	762	488	274
Nitrogen (Kjeldahl)	871	553	318
Nitrogen (Dumas)	1447	740	707
Carbon and hydrogen	1729	845	884
Halogen (Carius)	296	168	128
Sulfur (Carius)	184	120	64
Molecular weight	432	256	176
Total	6360	3600	2760

From the above figures another important fact may be observed, namely, that a course of this type, aside from its educational value, constitutes a definite asset to any institution pursuing organic chemical research, inasmuch as it affords the analysis of a large number of research substances.

The teaching schedule may be varied to suit individual needs. Thus it may be suitably shortened by either omitting analyses of research substances or by adapting one of the following shortened schedules or by a combination of both.

SHORTENED SCHEDULES

Periods	*A*	*B*
1st and 2nd	Weighing	Weighing
3rd	Determination of metals	Titrations
4th	Titrations	Kjeldahl
5th and 6th	Dumas	Dumas
7th, 8th and 9th	Carbon and hydrogen	Carbon and hydrogen
10th and 11th	Molecular weight (cryoscopic)	Halogen and sulfur

Remarks

With the introduction of ordinary analytical balances of proper sensitivity and precision in quantitative organic microanalysis [20] there appears to be less need than ever to resort to so-called semi-micromethods,[9, 14, 16, 23, 24] but the classical quantitative micro procedures of F. Pregl,[21, 22] F. Emich,[10, 11] and others [5, 12, 17, 19, 25] can be taught and practiced under conditions as found in any average quantitative chemical laboratory. A course in organic quantitative microanalysis has, in addition to the often-repeated benefits of a course in microtechnic,[1–4, 6–10, 13, 15, 18, 23] such advantages as development of "experimental asepsis," saving of time and materials, and this exceptional feature—that it is also of invaluable aid to organic chemical research. At present some fifteen universities and colleges in the United States have a course in quantitative organic microanalysis in their regular teaching curriculum.[4a]

LITERATURE

1. Abrahamczik, E., and Blümel, F., *Mikrochemie*, **24**, 268 (1938).
2. Anonymous, "Ausbildung fur mikrochemische Arbeiten," *Mikrochemie*, **22**, 265 (1937); (*a*) "New York University Centennial," *Mikrochemie*, **11**, 12 (1932).
3. Behrens, H., "A Manual of Microchemical Analysis," Macmillan Co., London and New York, 1894; (*a*) Behrens, H., and Kley, F., "Mikrochemische Analyse," L. Voss, Leipzig and Hamburg, 1915.
4. Benedetti-Pichler, A. A., and Spikes, W. F., "Introduction to the Microtechnique of Inorganic Qualitative Analysis," Microchemical Service, Douglaston, L. I., N. Y., 1935; (*a*) *Mikrochemie*, **22**, 267–271 (1937).
5. Boetius, M., "Über die Fehlerquellen bei der mikro-analytischen Bestimmung des Kohlen- und Wasserstoffes nach der Methode von Fritz Pregl," Verlag Chemie, Berlin, 1931.
6. Chamot, É. M., *J. Chem. Education*, **5**, 9, 258 (1928); *Ind. Eng. Chem., Anal. Ed.*, **4**, 7 (1932).
7. Chamot, É. M., and Mason, C. W., "Handbook of Chemical Microscopy," John Wiley & Sons, New York, 1931.
8. Clarke, B. L., *J. Chem. Education*, **14**, 561 (1937).
9. Engelder, C. J., and Schiller, W., *J. Chem. Education*, **9**, 1636 (1932).

10. Emich, F., "Mikrochemisches Praktikum," J. F. Bergmann, Munich, 1931; "Lehrbuch der Mikrochemie," J. F. Bergmann, Munich, 1926.
11. Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York, 1932.
12. Friedrich, A., "Die Praxis der quantitativen organischen Mikroanalyse," F. Deuticke, Leipzig and Vienna, 1933.
13. Garner, W., "Industrial Microscopy," I. Pitman & Sons, London, 1932.
14. Grassner, F., *Mikrochemie*, **10**, 255 (1932).
15. Grey, E. C., "Practical Chemistry by Micro-Methods," W. Heffer & Sons, Cambridge, 1925.
16. Gutzeit, G., *Helv. Chim. Acta*, **12**, 829 (1929).
17. Lieb, H., and Benedetti-Pichler, A. A., "Mikrochemische Analyse," Berl-Lunge, "Chemisch-technische Untersuchungsmethoden," Vol. I, Eighth Edition, J. Springer, Berlin (1931).
18. Mabee, F. C., *Rept. New England Assoc. Chem. Teachers*, **38**, 34 (1936).
19. Niederl, J. B., *J. Chem. Education*, **13**, 254 (1936).
20. Niederl, J. B., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **11**, 412 (1939).
21. Pregl, F., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935.
22. Roth, H., and Daw, E. B., "Quantitative Organic Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937.
23. Spoerri, T. E., *J. Chem. Education*, **10**, 491 (1933).
24. Ter Meulen, H., and Heslinga, J., "Neue Methoden der organisch-chemischen Analyse," Akademische Verlagsanstalt, Berlin, 1927.
25. Weygand, C., "Quantitative analytische Mikromethoden der organischen Chemie in vergleichender Darstellung," Akademische Verlagsgesellschaft, Leipzig, 1931.

II. INSTALLATION OF A LABORATORY FOR QUANTITATIVE ORGANIC ELEMENTARY MICROANALYSIS

General Layout

The general layout of a laboratory for quantitative organic elementary microanalysis intended for industrial use need not differ from one designed for teaching purposes. There are several reports in the literature citing the experiences in the installation of such laboratories.[1-20]

The laboratory for quantitative organic elementary microanalysis at New York University, Washington Square College, New York City, which is described below, has been in continuous use for teaching as well as research purposes since its installation in 1925.[3a, 16]

Laboratory. Two rooms are used, which are neither air conditioned, nor possess any dust, humidity, or temperature control. One room, one section of which serves as the balance room, measures 13½ by 29 feet; the other room, in which the various set-ups, with the exception of the Dumas apparatus, are located, is 29 feet long, its front section, which faces the windows, being 23 feet wide.

Balances. Four microanalytical balances (three Kuhlmann and one Starke-Kammerer) varying in sensitivity and precision from ±1 to ±3 micrograms, and two ordinary analytical balances (Ainsworth and Becker), possessing a sensitivity and precision of ±10 micrograms each, are set up on an L-shaped heavy stone table at the wall opposite the window. One section of the L-shaped stone table, which accommodates the two analytical balances and one microbalance, measures 9½ by 2½ feet, and the other section, providing accommodation for four microbalances, measures 13½ by 2½ feet. The only permanent set-up in this room is the double Dumas apparatus which, being near the window of the room, is well away from the balances. In the adjoining room are the double carbon and hydrogen combustion trains, the double Kjeldahl, the molecular-weight, and the halogen and sulfur apparatus. The center table, provided with adequate lighting, is reserved for the titration equipment, that is for the burets containing the various standard solutions. On the three tables flanking the center table, there are, in the front and facing the windows, the double carbon and hydrogen combustion trains, at the right the double Kjeldahl apparatus, and at the left the molecular-weight and halogen and sulfur apparatus. The free space left is used for the temporary set-ups. The double Dumas apparatus, as mentioned above, is located in the adjacent balance room.

Permanent Set-Ups

Carbon and Hydrogen Apparatus. This requires a table 8 feet in length and 2 feet in width. It can be an ordinary wooden table, but if gas burners are used its top should be of stone, or else asbestos sheets should be placed below the gas burners. The oxygen tank, well secured, is placed either on the side or back of the apparatus, so that it does not obstruct the movements of the operator in any way. For the aspirator, which should be located near the balance, a table 3 feet in length and 2 to 3 feet in width is used.

Kjeldahl Apparatus. A space of about 3 by 2 feet is necessary to accommodate the distillation apparatus and one steamer. Gas as well as water connections and a sink must be available. An additional space of about 2 feet each is required for the digestion stand and the titration outfit. Altogether a table of 7 by 2 feet is required.

Halogen and Sulfur Determination Apparatus. Any one of these apparatus requires a table 3 by 2 feet and an additional 3 by 2 feet for the water bath and filtration. The space where the filtration and drying are carried out is covered preferably with a glass plate. The table for the apparatus itself should have a stone top or be adequately protected from the heat of the burners by a heavy asbestos sheet.

Apparatus for the Molecular-Weight Determinations. None of the methods for the determination of molecular weight requires more than about 2 by 3 feet of table space.

Titration Apparatus. Three standard solutions are required:

0.01 N acid solution,
0.01 N sodium hydroxide solution,
0.01 N sodium thiosulfate solution.

Burets of 10-ml. capacity and calibrated to 0.05 ml. with automatic zero-point adjustment, and provided with protection tubes filled with Ascarite and a suitable automatic filling arrangement, are used. One-liter stand bottles are usually employed.

Total Space. A room 10 by 20 feet will provide sufficient space for the balance and all apparatus, as well as a small office desk. The minimum table space required for single set-ups would be about 34 by 2 feet, distributed as listed below:

3 by 2 feet for the balance
8 by 2 feet for the carbon and hydrogen apparatus
3 by 2 feet for the aspirator
6 by 2 feet for the Dumas apparatus
5 by 2 feet for the Kjeldahl apparatus
3 by 2 feet for the titration equipment
3 by 2 feet each for the halogen and sulfur apparatus
3 by 2 feet for each apparatus for the molecular weight determination

Temporary Set-Ups

Most of the apparatus employed in the quantitative determination of elements in organic compounds are permanent set-ups. Small or compact apparatus can be easily stored away on the side shelves or in drawers, either in the assembled or dismantled state (determination of metals, molecular weights, alkoxyl, etc.). Some of the apparatus for the determination of atomic or molecular groupings (active hydrogen, etc.) are rather complicated affairs and, if they are used constantly, a permanent set-up is certainly in order.

Cost

In order to find the minimum cost for the installation of the quantitative elementary organic microanalytical methods the catalogs and price lists of several commercial supply houses have been consulted. For single set-ups the prices varied from $1000 to $1200. This includes a microanalytical balance of the usual type and the weighing utensils as well as the miscellaneous laboratory utensils, the carbon and hydrogen apparatus, the Dumas and the Kjeldahl apparatus, one complete apparatus for the Carius method for the determination of halogen and sulfur, one each for the molecular-weight determination.

Personnel

The person in charge of a microanalytical laboratory practicing the methods described must not only be able to follow the various procedures, but must also be capable of setting up the various apparatus and of detecting and remedying defects. The taking of a course in quantitative organic elementary microanalysis alone will not suffice for the acquisition of the necessary knowledge. The additional experience required may be gained either by the trial-and-error method by the individual concerned, or by the far easier method of duplicating working set-ups thoroughly tried in a laboratory already adapted to the methods. The person performing the routine analytical work does not have to be a highly trained individual. The paramount requirement in routine analysis is the orthodox and unswerving following of acknowledged procedures, deviations from which usually end in unsatisfactory analytical results.

Applications and Reports of Analyses

At universities as well as in industrial or academic research organizations it is advisable, in order to save unnecessary work and to facilitate the execution of the analysis, that for every substance to be analyzed an application for analysis be made out which is signed or endorsed by the person in charge of the research. Such an application, as shown below, should state some of the physical and chemical characteristics of the compound. It should also give information in regard to the behavior of the substance upon combustion, that is, whether the compound is explosive, whether it sublimes, or whether it is hygroscopic, or volatile, or very difficult to combust. All these properties of the substance require special attention, and much time is saved by furnishing the analyst with this information, who otherwise may waste an analysis, during the course of which he has to acquaint himself with the behavior of the sample.

APPLICATION FOR ANALYSIS

Name of applicant.......................................Date................
Recommended by (director of research)..
Name or number of compound..
Physical constants of compound:
M.p.:..........B.p.:..........Other constants...........................
Criteria for the purity of the compound (state reasons for believing it is pure):
....
....
Behavior of compound on heating (state whether the substance distils, sublimes, decomposes, explodes, and whether easy or difficult to combust):
....
State whether compound is volatile..............hygroscopic.................
Quantitative analysis is desired for the following elements:
Carbon and hydrogen......................Nitrogen.........................
Halogen..................Sulfur..................Mol. wt.................
Other determinations...
(Samples of about 20 mg. in small vials or sealed ampules, well labeled, should be furnished; the samples will not be returned, but are kept for reference.)

Upon completion of the analysis a corresponding report is made out in duplicate by the analyst, one of which is retained by him, giving the weight of the sample, the weight or volume of the combustion products, and, for the carbon and hydrogen determination, a statement whether or not a residue was observed. A permanent record of all the analyses performed should be kept, because it is often necessary to look up the results of an analysis made months ago. An example of such a report is given below:

REPORT OF ANALYSIS

Name or number of compound.............................Date.................

I II

Quantitative carbon and hydrogen determination:

Weight of sample..
Weight of water...
Weight of carbon dioxide..................................
Percentage of hydrogen....................................
Percentage of carbon......................................

Quantitative nitrogen determination:

Weight of sample..
Net volume of nitrogen....................................
Temperature and pressure..................................
Percentage of nitrogen....................................

Other determinations:

Kind of determination.....................................
Weight of sample..
Weight of...
Milliliters of ....
Percentage of...

Molecular-weight determination:

Ebullioscopic...........Cryoscopic.....................
Weight of sample..
Weight of solvent...
Depression, elevation.....................................
Molecular weight..

Remarks..
Record book: Date................ Page.............. No..............
Analysis performed for...
Analyst.......................................

It is advisable that upon the receipt of the report the research worker advise the analyst whether the results checked with the expectation, particularly whether they were within the general limits of accuracy of the respective methods involved. The accuracy of the various methods is given in the table on p. 279, and from this table it is evident that no *micro* errors can be expected. Since the reaction laws do not change when the size of the sample is decreased, micromethods cannot generally be expected to give better results than the respective macromethods, although quite often in the hands of an experienced analyst results within ±0.1% of the theoretical value are obtained, with the exception of the structure analytical, and the molecular-weight methods.

When finer differentiation than the above-mentioned average limits of errors is required, it is by far the wiser procedure to resort to *structure analytical designing* than to demand a higher than usual precision from the analyst. By the introduction of suitable atomic or molecular groupings into the compound (halogen, nitro groups, carboxyl groups, etc.) much finer differentiation is possible than is obtainable by the sole use of analytical methods. The same holds true in the application of any of the structure microanalytical methods (alkoxyl, alkimide, acyl, active hydrogen, etc.). A survey of the literature will bring to light the surprising fact that practically every microanalytical laboratory has its own modifications of such methods. Systematic studies of such methods usually reveal that the procedures given work excellently for the examples cited, but fail, or at least require further modifications and improvements, when used for other types of compounds. With the exception of laboratories where these highly special methods are an absolute necessity and are applied to the same type of substances, it might prove of far greater expediency to resort again to the preparation of suitable derivatives than to force the analyst to set up complicated apparatus and spend one or several weeks in getting satisfactory blanks, only to find out later that the method does not apply to the compounds in question.

In conclusion it can be said that for an average organic research laboratory, even if a great variety of substances belonging to various orders are to be analyzed, the micromethods of quantitative organic elementary analysis given in this book should prove sufficient.

LIST OF CHEMICALS

- Acetic acid
- Acetone
- Ammonium
  - chloride
  - hydroxide
  - iodide
  - molybdate
  - nitrate
  - sulfate
- Amyl ether
- Anethole
- Anhydrone
- Aniline
- Asbestos
- Ascarite
- Barium
  - chloride
  - hydroxide
  - oxide
- Benzene
- Borneol
- Bromine
- Cadmium sulfate
- Camphor
- Copper
  - metallic
  - oxide, wire
  - sulfate
- *p*-Cymene
- Ether
- Ethyl alcohol
- Formic acid
- Glass cement
- *d*-Glucose
- Gold chloride
- Hydrazine sulfate
- Hydrochloric acid
  - (sp. gr.: 1.18)
- Hydriodic acid
  - (sp. gr.: 1.7, 1.96)
- Iodine
- Lead peroxide
- Magnesium, metal
- Marble

Mercury
  acetate
  metal
Methyl red
Nitric acid
  (sp. gr.: 1.42)
Nitrogen, tank
Oxygen, tank
Perhydrol (30%)
Phenol
Phenolphthalein
Phosphorus, red
Platinized asbestos
Platinum
  contacts
  gauze
  tetrahedrons
  wire
Potassium
  biiodate
  chlorate
  cyanide
  dichromate
  hydroxide
  iodate
  iodide
  metal
  nitrate
  sulfate
Propionic acid anhydride
Pyridine
Silver
  dichromate
  nitrate
  wire
  wool
Sodium
  acetate
  bisulfite
  carbonat
  hydroxide
  metal
  nitrate
  peroxide
  thiosulfate
Starch
Stopcock grease
Sulfuric acid (sp. gr.: 1.84)
p-Toluene sulfonic acid

LITERATURE

1. Abrahamczik, E., and Blümel, F., *Mikrochemie,* **24**, 268 (1938).
2. Alber, H. K., and Harand, J., *J. Franklin Inst.*, **224**, 729 (1937); *Ind. Eng. Chem., Anal. Ed.*, **10**, 403 (1938).
3. Anonymous, "Ausbildung für mikrochemische Arbeiten," *Mikrochemie,* **22**, 265 (1937); (*a*) "New York University Centennial," *Mikrochemie,* **11**, 12 (1932).
4. Brown, J. W., *Can. Chem. Met.*, **16**, 1 (1932).
5. Clarke, B. L., *Ind. Eng. Chem.*, **23**, 1301 (1931).
6. Clarke, B. L., and Hermance, H. W., *Ind. Eng. Chem., Anal. Ed.*, **7**, 218 (1935).
7. Dubsky, J. V., *Chem. Weekblad,* **34**, 599 (1937).
8. Gicklhorn, J., *Mikrochemie,* **11**, 369 (1932).
9. Grassner, F., *Mikrochemie,* **10**, 257 (1932).
10. Kirk, P. L., *Am. Rev. Biochem.*, **6**, 73 (1937).
11. Kirner, W. R., *Ind. Eng. Chem., Anal. Ed.*, **5**, 363 (1933); **9**, 300 (1937).
12. Lieb, H., *Mikrochemie,* **10**, 230 (1931).
13. Lieb, H., and Soltys, A., *Mikrochemie, Molisch Festschrift,* 1937, p. 290.
14. Lindenfeld, K., *Mikrochemie,* **16**, 153 (1935).
15. McDonald, E., *J. Franklin Inst.*, **225**, 164 (1938).
16. Niederl, J. B., *Ind. Eng. Chem., Anal. Ed.*, **7**, 214 (1935); *J. Chem. Education,* **13**, 254 (1936).
17. Peterson, J. B., and Schöffel, E. W., Rochester Meeting, Am. Chem. Soc., September 9, 1937; *Ind. Eng. Chem., Anal. Ed.*, **10**, 172 (1938).
18. Shelberg, E. F., *Ind. Eng. Chem., Anal. Ed.*, **10**, 704 (1938).
19. Tiedcke, C., *Mikrochemie,* **25**, 65 (1938); *Mikrochemie-Mikrochim. Acta,* **25**, 399 (1938).
20. Van Nieuwenburg, C. J., *Mikrochemie,* **21**, 184 (1936); *Chem. Listy,* **31**, 38 (1937).

III. QUALITATIVE ORGANIC ANALYSIS

In the course of teaching *qualitative organic analysis* for the last thirteen years in the Graduate School of New York University at both the University Heights and the Washington Square branches,[74, 81a] several attempts have been made to introduce micromethods instead of the conventional macro procedures. It was found that it would not be possible to maintain, for every term, the present working schedule of analyzing seventeen unknowns, including two mixtures of three components each, if micro procedures were used exclusively.

Even though present-day qualitative organic microchemical methods [81] may not lend themselves very readily to routine teaching within the average graduate chemistry curriculum, nevertheless they have a definite place in organic chemical research when small amounts of material are involved. Thus in the following a brief literature survey is presented of present-day microchemical methods for the isolation, purification, and identification of organic compounds.

Isolation and Purification. The microprocedures of isolation and purification [23, 30e, 105, 109] of organic compounds are usually modeled after the corresponding macro procedures. They comprise *extraction,*[6, 13, 14a, 20, 22a, 26a, 32, 38, 40, 41, 42, 47, 53, 58, 70–**72**, **81**b, 83, 102, 106, 108, 115] *distillation,*[8d, 9, 16, 22, 30b, 31c, 33, 43] *sublimation,*[8e, 16, 30c, 31e, 76a, 97, 101] and *chromatographic* procedures.[113, 116, 117] For the *crystallization* and *recrystallization* of solids, depending upon the amount of material available, small beakers, test tubes, and centrifuge cones of various sizes, or capillaries, are used.[1, 9, 31a, b, 86, 91, 97] Instead of filtration,[68, 86, 90, 91] centrifuging and the use of *porous tiles* is preferable.

Ordinal Tests.[79] The micromethods for the detection of elements in organic compounds [5, 26, 30f, 31f, 36, 49, 111, 112] include tests for carbon and hydrogen, nitrogen, halogen, sulfur, and other elements. Most useful of these tests is the *micro ammonia* test as devised by F. Emich.[30f, 31f]

Generic Tests. The literature abounds in the description of microchemical tests with and without the use of a microscope [1, 4, 7, 30g, j, 31g, 36, 41, 48, 63, 76, 95, 107, 114] for the various types of organic compounds. These methods usually involve color reactions, hydrolysis procedures, or the preparation of suitable derivatives for identification purposes. In the majority of cases it usually suffices to employ well-known standard macro procedures and tables,[15, 18, 28, 51, 54, 55, 59, 60, 69, 75, 79, 84, 85, 89, 96, 98, 103, 111] and, depending on the amount of substance available, small distillation flasks and reflux apparatus, test tubes, both open and sealed, centrifuge cones of various sizes, and sealed capillaries have found application.

Determination of Physical Constants. The conventional *melting-point* determination is itself a micro procedure, which may be augmented and refined by using a polarizing microscope with a suitably constructed hot stage.[8c, 24, 27, 61, 64, 65–67, 86a, 91a, 110, 118] For the determination of the *boiling point* a large variety of methods exists;[8d, 30b, 31c, 43–45, 49, 76, 82, 86b, 88, 91b, 94, 100] of these, the method of A. Siwoloboff[79a, 100] appears to be the most practical.

Solubility tests are carried out with centrifuge cones, with capillaries, on microscope slides,[16b, 62, 95] or by means of *schlieren.* Quite a variety of procedures including the *schlieren* method[1d, 3, 30l, 31e, 93] have been devised for the determination of the *specific gravity*[1a, 8a, 10–12, 14, 16a, 17, 19, 21, 25, 30d, 34, 35, 37, 39, 46, 52, 73, 77, 86d, 87, 91d, 104] and the *refractive index*[1b, c, 2, 8f, 16a, 29, 30a, 56, 57, 62, 65b, 72, 80, 86c, e, 91c, 92, 93, 99, 114] of both liquid and solid organic substances and solutions. Micromethods for the determination of *viscosity*[8b, 78] and *surface tension* are also known.[8b]

In addition to the above-cited typical micro procedures, the methods which have been actually used in the isolation, purification, identification, and synthesis of certain rare alkaloids, the hormones, and the vitamins should also be consulted. Thus, in a combination of methods, rather than in the orthodox following of micro procedures alone, lies the solution of many an organic research problem.

LITERATURE

1. Alber, H. K., "Mikrokemi," P. A. Norstedt & Soners, Stockholm, 1933; (*a*) *Ind. Eng. Chem., Anal. Ed.*, **12**, 764 (1940); (*b*) *J. Franklin Inst.*, **226**, 813 (1938); (*c*) *Mikrochemie*, **18**, 92 (1935); **25**, 167 (1938); (*d*) *Z. anal. Chem.*, **90**, 87 (1932).
2. Alber, H. K., and Bryant, J. T., *Ind. Eng. Chem., Anal. Ed.*, **12**, 305 (1940).
3. Alber, H. K., and Renzenberg, M., *Z. anal. Chem.*, **86**, 114 (1931).
4. Allen, R. M., "The Microscope," D. Van Nostrand Co., New York, 1940.
5. Baker, R. H., and Barkenbus, C., *Ind. Eng. Chem., Anal. Ed.*, **9**, 135 (1937).
6. Batt, W. G., and Alber, H. K., *Ind. Eng. Chem., Anal. Ed.*, **13**, 127 (1941).
7. Behrens, H., and Kley, F., "Organische mikrochemische Analyse," Hamburg and Leipzig, 1922.
8. Benedetti-Pichler, A. A., "Die Fortschritte der Mikrochemie in den Jahren 1915 bis 1926," G. Klein and R. Strebinger, Vienna, 1927, (*a*) p. 164; (*b*) p. 172; (*c*) p. 175; (*d*) p. 176; (*e*) p. 178.

9. Benedetti-Pichler, A. A., and Spikes, W. F., "Introduction to the Microtechnique of Inorganic Qualitative Analysis," Microchemical Service, Douglaston, L. I., 1935.
10. Bernal, J. D., and Crowfoot, D., *Nature*, **134**, 809 (1934); **135**, 305 (1935).
11. Beutler, R., *Mikrochemie*, **16**, 133 (1935).
12. Blank, E. W., and Willard, M. L., *J. Chem. Education*, **10**, 109 (1933).
13. Blount, B. K., *Mikrochemie*, **19**, 162 (1936).
14. Browning, B. L., *Paper Trade J.*, **105**, No. 10, 38 (1937); *Mikrochemie*, **26**, 55 (1939); (*a*) *Tech. Assoc. Papers*, **20**, 311 (1937).
15. Campbell, N., "Qualitative Organic Chemistry," D. Van Nostrand Co., New York, 1939.
16. Chamot, E. M., and Mason, C. W., "Handbook of Chemical Microscopy," John Wiley & Sons, New York, 1931, (*a*) Vol. I, p. 364; (*b*) Vol. II, p. 4.
17. Ciochina, J., *Z. anal. Chem.*, **107**, 108 (1936).
18. Clarke, H. T., "A Handbook of Organic Analysis," E. Arnold & Co., Fourth Edition, London, 1926.
19. Clemo, G. R., and McQuillen, A., *J. Chem. Soc.*, **1935**, 1220.
20. Colegrave, E. B., *Analyst*, **60**, 90 (1935).
21. Collander, R., *Protoplasma*, **8**, 440 (1929).
22. Craig, L. C., *Ind. Eng. Chem., Anal. Ed.*, **8**, 219 (1936); (*a*) **12**, 773 (1940).
23. Dadieu, A., and Kopper, H., *Z. angew. Chem.*, **50**, 367 (1937).
24. Dennis, L. M., and Shelton, R. S., *J. Am. Chem. Soc.*, **52**, 3128 (1930).
25. Derner, V. H., and Derner, O. C., *J. Am. Chem. Soc.*, **59**, 1148 (1937).
26. Donau, J., *Monatsh.*, **29**, 333 (1908); (*a*) **60**, 129 (1932).
27. Dunbar, R. E., *Ind. Eng. Chem., Anal. Ed.*, **11**, 516 (1939).
28. Eastman Kodak Co., "Eastman Organic Chemicals," 1940.
29. Edwards, A. E., and Otto, C. E., *Ind. Eng. Chem., Anal. Ed.*, **10**, 225 (1938).
30. Emich, F., "Lehrbuch der Mikrochemie," Second Edition, J. F. Bergmann, Munich, 1926; (*a*) p. 26; (*b*) p. 51; (*c*) p. 56; (*d*) p. 105; (*e*) p. 119; (*f*) p. 191; (*g*) p. 202; (*h*) p. 232; (*i*) p. 257; (*j*) p. 228; (*k*) *Monatsh.*, **38**, 219 (1917); (*l*) **50**, 269 (1928); **53/54**, 312 (1929).
31. Emich, F., and Schneider, F., "Microchemical Laboratory Manual," John Wiley & Sons, New York; (*a*) p. 12; (*b*) p. 29; (*c*) p. 32; (*d*) p. 39; (*e*) p. 40; (*f*) p. 112; (*g*) p. 118.
32. Erdös, J., and Pollak, L., *Mikrochemie*, **19**, 245 (1936).
33. Fife, I. M., *Ind. Eng. Chem., Anal. Ed.*, **8**, 316 (1936).
34. Fischer, E., *Ber.*, **44**, 129 (1911).
35. Flaschenträger, B., *Z. angew. Chem.*, **39**, 717 (1926).
36. Foulke, O. G., and Schneider, F., *Ind. Eng. Chem., Anal. Ed.*, **10**, 104 (1938); **12**, 554 (1940).
37. Francis, L. D., *J., Lab. Clin. Med.*, **22**, 718 (1937).
38. Fulton, R. W., *Ind. Eng. Chem., Anal. Ed.*, **9**, 437 (1937).
39. Furter, M., *Helv. Chim. Acta*, **21**, 1666, 1680 (1938).
40. Gagarin, R., *Chem. Ztg.*, **51**, 204 (1933).
41. Garner, W., *Ind. Chemist*, **3**, 289 (1928); **5**, 58 (1928).
42. Gettens, R. J., *Tech. Studies Field of Fine Arts*, **2**, 107 (1933).
43. Gettler, A. O., Niederl, J. B., and Benedetti-Pichler, A. A., *J. Am. Chem. Soc.*, **54**, 1476 (1932); *Mikrochemie*, **11**, 167 (1932).
44. Gettler, A. O., and Fine, J., *Ind. Eng. Chem., Anal. Ed.*, **10**, 449 (1938).
45. Gettler, A. O., *Ind. Eng. Chem., Anal. Ed.*, **11**, 559 (1939).

46. Gill, A. H., and Simms, H. S., *Ind. Eng. Chem.*, **13**, 547 (1921).
47. Gorbach, G., *Mikrochemie*, **12**, 161, 189 (1932).
48. Griebel, C., and Weiss, F., *Mikrochemie*, **5**, 146 (1927).
49. Hayman, D. F., *Ind. Eng. Chem., Anal. Ed.*, **11**, 470 (1939).
50. Hays, E. E., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **8**, 286 (1936).
51. Heilbron, I. M., "Dictionary of Organic Compounds," Vols. I, II, and III, Oxford University Press, New York, 1934.
52. Hendricks, S. B., and Jefferson, M. E., *J. Optical Soc. Am.*, **23**, 299 (1933).
53. Hetterich, H., *Mikrochemie*, **10**, 379 (1932).
54. Huntress, E. H., "A Brief Introduction to the Use of Beilstein's Handbuch der organischen Chemie," John Wiley & Sons, New York, 1938.
55. Huntress, E. H., and Mulliken, S. P., "Identification of Pure Organic Compounds," John Wiley & Sons, New York, N. Y., 1941.
56. *International Critical Tables*, Vol. I, pp. 276–306.
57. Jelley, E. E., *J. Roy. Microscop. Soc.*, **54**, 234 (1934).
58. Jendrassik, L., and Petras, P., *Biochem. Z.*, **226**, 381 (1930).
59. Kamm, O., "Qualitative Organic Analysis," John Wiley & Sons, New York, 1932.
60. Kempf, R., and Kutter, F., "Schmelzpunkt Tabellen zur organischen Molekularanalyse," F. Vieweg und Sohn, Braunschweig, 1928.
61. Kempf, R., "Die Methoden der organischen Chemie," Third Edition, J. Houben, 1925, Vol. I, p. 797.
62. Kirk, P. L., and Gibson, G. L., *Ind. Eng. Chem., Anal. Ed.*, **11**, 403 (1939).
63. Kisser, J., and Kondo, Y., *Mikrochemie, Molisch Festschrift*, 1936, p. 259.
64. Klein, G., *Mikrochemie, Pregl Festschrift*, 1929, p. 192.
65. Kofler, L., *Arch. Pharmaz.*, **270**, 293 (1932); (*a*) *Mikrochemie*, **15**, 242 (1934); (*b*) **22**, 241 (1937); (*c*) "Mikroskopische Methoden zur Identifizierung organischer Substanzen," Verlag Chemie, Berlin, 1940.
66. Kofler, L., and Hilbck, H., *Mikrochemie*, **9**, 38 (1931).
67. Kofler, L., and Ruess, H., *Chem. Erde*, **11**, 590 (1938).
68. Kolthoff, I. M., and Amdur, E., *Ind. Eng. Chem., Anal. Ed.*, **12**, 178 (1940).
69. Lange, N. A., "Handbook of Chemistry," Handbook Publishers, Sandusky, O., 1940.
70. Lehrecke, H., *Z. angew. Chem.*, **49**, 620 (1936).
71. Mayrhofer, A., "Microchemie der Arzneimittel und Gifte," Berlin and Vienna, 1923.
72. Mayrhofer, A., and co-workers, *Pharm. Presse, Wiss. prakt. Heft*, **37**, 130 (1932); *Scientia Pharm.*, **5**, 105 (1934); *Pharm. Monatsh.*, **17**, 41 (1936).
73. McLean, and Adams, R., *J. Am. Chem. Soc.*, **58**, 804 (1936).
74. McGreal, M. E., and Niederl, J. B., Cincinnati Meeting, Am. Chem. Soc., April, 1940.
75. Middleton, H., "Systematic Qualitative Organic Analysis," E. Arnold & Co., London, 1939.
76. Morton, A. A., and co-workers, *Ind. Eng. Chem., Anal. Ed.*, **5**, 185 (1933); **11**, 460 (1939); **13**, 494, 498, (1941).
77. Mouquin, H., and Alber, H. K., *Mikrochemie*, **21**, 142 (1936).
78. Mouquin, H., and Natelson, S., *J. Phys. Chem.*, **35**, 1931 (1931).
79. Mulliken, S., "A Method for the Identification of Pure Organic Compounds," Vols. I, II, III, and IV, John Wiley & Sons, New York, 1904; (*a*) Vol. I, p. 222.
80. Nichols, L., *Natl. Paint Bull.*, **1**, 5, 12, 14 (1937).

81. Niederl, J. B., *Ind. Eng. Chem., Anal. Ed.*, **7**, 214 (1935); (*a*) Chemistry 201: Semi-micro Qualitative Organic Analysis, New York University, N. Y., 1941; (*b*) *J. Am. Chem. Soc.*, **51**, 474 (1929).
82. Niederl, J. B., and Routh, I. B., *Mikrochemie*, **11**, 251 (1932).
83. Noyons, E., *Chem. Weekblad*, **30**, 228 (1933).
84. Prager, B., and Jacobson, P., "Beilstein's Handbuch der organischen Chemie," J. Springer, Berlin, 1918; (*a*) acyclic series, Vols. I–IV; (*b*) isocyclic series, Vols. V–XVI; (*c*) heterocyclic series, Vols. XVII–XXVII.
85. Prager, B., Stern, D., and Ilberg, K., "System der organischen Verbindungen," J. Springer, Berlin, 1929.
86. Pregl, F., "Die quantitative organische Mikroanalyse," Fourth Edition, J. Springer, Berlin, 1935; (*a*) p. 270; (*b*) p. 273; (*c*) p. 306; (*d*) p. 307; (*e*) *Fermentforschung*, **2**, 63 (1917).
87. Retgers, J. W., *Neues Jahrb. Mineral. Geol.*, **1889** (2) 185; *Z. physik. Chem.*, **3**, 298, 497 (1889).
88. Rosenblum, C., *Ind. Eng. Chem., Anal. Ed.*, **10**, 449 (1938).
89. Rosenthaler, L., "Der Nachweis organischer Verbindungen," F. Enke, Stuttgart, 1923; *Mikrochemie*, **8**, 72 (1930).
90. Roswell, C. A., *Ind. Eng. Chem., Anal. Ed.*, **12**, 350 (1940).
91. Roth, H., and Daw, E. B., "Quantitative Microanalysis of Fritz Pregl," P. Blakiston's Son & Co., Philadelphia, Pa., 1937; (*a*) p. 222; (*b*) p. 225; (*c*) p. 253; (*d*) p. 254.
92. Saylor, C. P., *J. Research Natl. Bur. Standards*, **15**, 277 (1935).
93. Schally, E., *Monatsh.*, **58**, 399 (1931).
94. Schleiermacher, A., *Ber.*, **24**, 944 (1891).
95. Schneider, F., and Foulke, G. G., *Ind. Eng. Chem., Anal. Ed.*, **10**, 445 (1938).
96. Schoorl, N., "Organische Analyse," Third Edition, D. B. Centen, Amsterdam, 1937.
97. Shead, A. C., *Ind. Eng. Chem., Anal. Ed.*, **9**, 496 (1937).
98. Shriner, R. L., and Fuson, R. C., "The Systematic Identification of Organic Compounds," John Wiley & Sons, New York, 1935.
99. Simms, H. S., *Ind. Eng. Chem.*, **13**, 546 (1921).
100. Siwoloboff, A., *Ber.*, **19**, 795 (1886).
101. Soltys, A., *Mikrochemie, Emich Festschrift*, 1930, p. 276.
102. Sperry, W. M., *Mikrochemie*, **12**, 151 (1933).
103. Staudinger, F., "Anleitung zur organischen qualitativen Analyse," J. Springer, Berlin, 1929.
104. Struszynski, M., *Przemysl Chem.*, **20**, 51 (1936).
105. Tamura, K., *Chem. Rev. (Japan)*, **3**, 396 (1937).
106. Titus, L., and Meloche, V. W., *Ind. Eng. Chem., Anal. Ed.*, **5**, 286 (1933).
107. Wagenaar, M., *Pharm. Weekblad*, **70**, 1029 (1933).
108. Wasitzky, A., *Mikrochemie*, **11**, 1 (1932).
109. Weston, P. E., *Ind. Eng. Chem., Anal. Ed.*, **5**, 179 (1933).
110. Weygand, C., and Grüntzig, W., *Mikrochemie*, **10**, 1 (1932).
111. Wilson, C. L., "An Introduction to Microchemical Methods," Methuen & Co., London, 1938; (*a*) *Analyst*, **63**, 332 (1938); **65**, 405 (1940).
112. Wilson, D. W., and Wilson, C. L., *J. Chem. Soc.*, **1939**, 1956.
113. Willstätter, R., and co-workers, *Ber.*, **55**, 3611 (1922); **57**, 1491 (1924).
114. Wright, F. E., *J. Wash. Acad. Sci.*, **4**, 269 (1914).

115. Wormley, T. G., "Microchemistry of Poisons," J. B. Lippincott, Philadelphia, Pa., 1867.

116. Zechmeister, L., "Die chromatographische Adsorptionsmethode," Second Edition, J. Springer, Berlin, 1938.

117. Zechmeister, L., and Cholnoky, L., "Principles and Practice of Chromatography," Chapman & Hall, London, 1940.

118. Zscheile, F. P., and White, J. W., *Ind. Eng. Chem., Anal. Ed.*, **12**, 436 (1940).

CALCULATIONS

STANDARD FORMULAS

A. Gravimetric Determinations

$$\%E = \frac{\text{wt. } E}{\text{wt. } s} \times 100 \text{ or } \frac{\text{wt. } E \text{ cpd.} \times f_{gr}}{\text{wt. } s} \times 100$$

B. Volumetric Determinations

$$\%E = \frac{\text{ml. std. sol.} \times f_v}{\text{wt. } s} \times 100$$

C. Gasometric Determinations

$$\%E = \frac{\text{ml. } v \times f_g}{\text{wt. } s} \times 100$$

$\%E$ = percentage element;

wt. s = weight of sample;

wt. E = weight of element;

wt. E cpd. = weight of element compound (CO_2; H_2O; $MeSO_4$; AgX; etc.);

ml. std. sol. = ml. standard volumetric solution used in the titration;

ml. v. = net volume of E;

f_{gr} (factor) $= \dfrac{\text{at. wt. } E}{\text{mol. wt. } E \text{ cpd.}}$;

f_v = equivalent weight of E per 1 ml. standard volumetric solution;

f_g = weight of 1 ml. E at the temperature and pressure of the gas vapors.

A. GRAVIMETRIC DETERMINATIONS

Type of Determination	*Element or Radical*	*Determined as*	*Factor*	*Log of Factor*
I. Metals	Ba	$BaSO_4$	0.5883	76972
	Ca	$CaSO_4$	0.2944	46894
	K	K_2SO_4	0.4487	65199
	Na	Na_2SO_4	0.3238	51025
V. Carbon and hydrogen	C	CO_2	0.2729	43600
	H	H_2O	0.1119	04875
VI. Halogen	Cl	AgCl	0.2474	39340
	Br	AgBr	0.4255	62890
	I	AgI	0.5405	73284
VII. Sulfur	S	$BaSO_4$	0.1373	13782
VIII. Phosphorus	P	$(MH_4)_3PO_4 \cdot 12MoO_3$ (empirical)	0.01453	16209
IX. Arsenic	As	$MgAs_2O_7$	0.4827	68368
S*—I. Alkoxyl and alkimide	OCH_3	AgI	0.1321	12096
	OC_2H_5	AgI	0.1918	28287
	CH_3	AgI	0.06398	80604
	C_2H_5	AgI	0.12380	09273

* S = structure analysis.

B. VOLUMETRIC DETERMINATIONS

Type of Determination	*Element or Radical*	*Standard Solution*	*Equivalent Weight of 1 ml., mg.*	*Log of Factor*
II. Neutralization equivalent (carboxyl)	COOH	0.01 *N* NaOH	0.4501	65331
III. Aminoid nitrogen	N	0.01 *N* acid	0.14008	14638
VI. Halogens	Cl	0.01 *N* NaOH	0.3546	54970
	Br	0.01 *N* NaOH	0.7992	90263
	I	0.01 *N* $Na_2S_2O_3$	0.21165	32562
VII. Sulfur	S	0.01 *N* NaOH	0.1603	20493
VIII. Phosphorus	P	0.1 *N* NaOH	0.1107	04415
IX. Arsenic	As	0.01 *N* $Na_2S_2O_3$	0.3748	57380
S—I. Alkoxyl and alkimide	OCH_3	0.01 *N* $Na_2S_2O_3$	0.05172	71366
	OC_2H_5	0.01 *N* $Na_2S_2O_3$	0.07510	87564
	CH_3	0.01 *N* $Na_2S_2O_3$	0.02503	39863
	C_2H_5	0.01 *N* $Na_2S_2O_3$	0.04839	68476
S—II. Acetyl	CH_3CO	0.01 *N* $Na_2S_2O_3$	0.4302	63370
Benzoyl	$C_6H_5CO—$	0.01 *N* $Na_2S_2O_3$	1.0504	02135

C. GASOMETRIC DETERMINATIONS

IV. Nitrogen (Dumas method)

ml. v (net volume of nitrogen collected)

Collected ml. of nitrogen (nitrometer reading);
Minus calibration correction (see nitrometer certificate);
Minus air error (determined by blank analysis);
Minus 1.1% of total collected ml. of nitrogen (0.5% for adhesion of the 50% potassium hydroxide solution to the walls, 0.3% for the vapor pressure of the 50% potassium hydroxide solution, 0.3% for the temperature reduction of the barometric reading from room temperature to 0° C.).

S—III. Active Hydrogen

Three separate calculations are necessary: (1) ml. v_t; (2) ml. v_T; (3) ml. v_f.

1. ml. $v_t = r_1 - v_r - v_b$.
2. ml. $v_T = r_2 - v_r - v_b$.
3. ml. $v_f = r_3 - r_2 - v_a$.

v_t = volume CH_4 at room temperature (t).
v_T = volume CH_4 at elevated temperature (T).
v_f = volume CH_4 at termination of analysis (final volume).
v_a = volume aniline added (reading of b_1).
v_b = volume CH_4 obtained in the blank test.
v_r = volume of reagent added (reading of b_2).
r_1 = first reading of methane buret (b_4).
r_2 = second reading of methane buret (b_4).
r_3 = third reading of methane buret (b_4).

For the calculation of the net volume of methane, as well as of hydrogen, the nitrogen reduction tables can be used by adding the respective factors:

$$CH_4 = \frac{22.4}{28} \text{ (log: 90309)} \qquad H = \frac{1}{28} \text{ (log: 55673)}$$

D. MOLECULAR-WEIGHT DETERMINATIONS

Indirect Methods

(*a*) From the percentage of element (metals, N, Hal., S, H, etc.):

$$M = \frac{\text{at. wt. } E}{\% E} \times 100$$

(*b*) From the percentage of a radical (R = COOH, CH_3O, C_2H_5O, CH_3CO, etc.):

$$M = \frac{\text{mol. wt. } R}{\% R} \times 100$$

If more than one atom equivalent of the element, or molecular equivalent of the radical (neutral salts of polybasic acids, dimethoxy compounds, etc.), is present, the result is a submultiple of the molecular weight.

(*c*) From the neutralization equivalent (N.E.):

$$M = \text{N.E.} \times y$$

y = number of free carboxyl groups present.

$$\text{N.E.} = \frac{\text{wt. } s}{\text{ml. std. sol.}}$$

The neutralization equivalent is equal to the molecular weight for a monobasic acid ($y = 1$); otherwise it is a submultiple, from which the molecular weight can be calculated by multiplying the neutralization equivalent with the number of free carboxyl groups present (N.E. $\times\ y$).

Direct Methods

(*a*) Solution methods (ebullioscopic and cryoscopic):

$$M = \frac{C \times \text{wt. } s}{\text{wt. } S \times \Delta}$$

C = constant of solvent (ebullioscopic method; molecular boiling-point elevation constant; cryoscopic method; molecular freezing-point depression constant).
wt. s = weight of sample (solute)
wt. S = weight of the solvent (in the ebullioscopic methods):
wt. S = sp. gr.$_s$ × 1.5 (Pregl method)
wt. S = sp. gr.$_s$ × 4.0 (Rieche method)
Δ = observed elevation in boiling point, or observed depression in the melting (or congealing) point of the solvent.

Practical Constants of Liquid Solvents (S)

	B.pt., °C.	Spec. grav.$_s^{20}$	$\frac{C}{1000\text{ g.}}$	Log of C
Acetic acid (glacial)......	118.1	1.049	3.07	48714
Acetone................	56.1	0.792	1.73	23805
Benzene................	80.5	0.879	2.57	40993
Chloroform..............	61.2	1.483	3.88	58883
Diethyl ether............	34.6	0.714	2.16	23445
Ethyl alcohol............	78.3	0.789	1.2	07918
Water..................	100.0	0.998	0.5	69897

Practical Constants of Solid Solvents (S)

	M. pt., °C.	$\frac{C}{1000\text{ g.}}$	Log of C
Borneol............................	206	35.8	55388
Camphene..........................	49	31.1	49276
Camphor...........................	176	40.0	60206
Pinene dibromide.....................	170	80.9	90795

(*b*) Vaporimetric method:

$$M = 62351\,\frac{\text{wt. } s\ (273.2 + T_2)}{VP}$$

$$V = \frac{g - c_1(T_2 - T_1)}{d} - vs$$

$$P = p_1 + p_2 - p_3 + p$$

wt. s = weight of sample.
V = volume of vapor.
g = weight of displaced mercury.
c_1 = correction for expansion per 1° C.
T_1 = initial temperature, °C.
T_2 = final temperature, °C.
d = density of mercury at T_2.
vs = volume of the sample $\frac{(\text{weight})}{(\text{density})}$ (approximately 1 cu. mm. per mg.)
P = pressure of vapor.
p_1 = barometer reading.
p_2 = vertical distance in millimeters between the mercury meniscus in the vaporizer and the orifice of the capillary outlet tube.
p_3 = vapor pressure of mercury at T_2.
p = capillary depression of mercury in outlet tube (+8 mm.); temperature reduction of barometer (−2 mm. at 15° C. − 4 mm. at 32° C.); density reduction of mercury in stem of vaporimeter which is inside of heating chamber (−1 mm. for T_2 = 100° C., −2 mm. for T_2 = 180° C., −3.5 mm. for T_2 = 320° C.).

The net value of p is usually small (about + 0.5% of P), and within the limits of accuracy of this method; therefore, for practical purposes this correction may be omitted.

Practical Constants—Using the High-Temperature Apparatus

Bath liquids: Water (100° C.).
p-Cymene (180° C.).
α-Naphthyl methyl ether (270° C.).
Benzyl benzoate (320° C.).
n-Butyl phthalate.

Mercury constants:			
d at	100° C.:	13.352	log: 12555
	180° C.:	13.160	11926
	270° C.:	12.947	11227
	320° C.:	12.829	10823
p_3 at	100° C.:	0 mm.	
	180° C.:	9 mm.	
	270° C.:	123 mm.	
	320° C.:	368 mm.	

Practical Constants—Using Water as a Heating Bath

Blank test with the apparatus: $B = \frac{g}{T_2 - T_1}$ = (c.g., 0.03)

$$M = 62351 \frac{\text{wt. } 373.2}{VP}$$

$$V = \frac{g - B(100 - T_1)}{13.352}$$

$$P = p_1 + p_2$$

(c) Isothermic method:

$$M = \frac{c \times 10}{m}$$

c = concentration of the sample solution.
m = molarity of the standard solution in isopiestic state with the sample solution.

NITROGEN REDUCTION TABLE *

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at t° and p mm.

t°	p=690	691	692	693	694	t°	p=695	696	697	698	699
10	03 946	04 009	04 072	04 135	04 197	10	04 260	04 322	04 385	04 447	04 509
11	03 793	03 856	03 919	03 982	04 044	11	04 107	04 169	04 232	04 294	04 356
12	03 640	03 703	03 766	03 829	03 891	12	03 954	04 016	04 079	04 141	04 203
13	03 488	03 551	03 614	03 677	03 739	13	03 802	03 864	03 927	03 989	04 051
14	03 336	03 399	03 462	03 525	03 587	14	03 650	03 712	03 775	03 837	03 899
15	03 184	03 247	03 310	03 373	03 435	15	03 498	03 560	03 623	03 685	03 747
16	03 033	03 096	03 159	03 222	03 284	16	03 347	03 409	03 472	03 534	03 596
17	02 883	02 946	03 009	03 072	03 134	17	03 197	03 259	03 322	03 384	03 446
18	02 733	02 796	02 859	02 922	02 984	18	03 047	03 109	03 172	03 234	03 296
19	02 584	02 647	02 710	02 773	02 835	19	02 898	02 960	03 023	03 085	03 147
20	02 435	02 498	02 561	02 624	02 686	20	02 749	02 811	02 874	02 936	02 998
21	02 287	02 350	02 413	02 476	02 538	21	02 601	02 663	02 726	02 788	02 850
22	02 139	02 202	02 265	02 328	02 390	22	02 453	02 515	02 578	02 640	02 702
23	01 992	02 055	02 118	02 181	02 243	23	02 306	02 368	02 431	02 493	02 555
24	01 846	01 909	01 972	02 035	02 097	24	02 160	02 222	02 285	02 347	02 409
25	01 700	01 763	01 826	01 889	01 951	25	02 014	02 076	02 139	02 201	02 263
26	01 555	01 618	01 681	01 744	01 806	26	01 869	01 931	01 994	02 056	02 118
27	01 410	01 473	01 536	01 599	01 661	27	01 724	01 786	01 849	01 911	01 973
28	01 266	01 329	01 392	01 455	01 517	28	01 580	01 642	01 705	01 767	01 829
29	01 122	01 185	01 248	01 311	01 373	29	01 436	01 498	01 561	01 623	01 683
30	00 979	01 042	01 105	01 168	01 230	30	01 293	01 355	01 418	01 480	01 540
31	00 836	00 899	00 962	01 025	01 087	31	01 150	01 212	01 275	01 337	01 397
32	00 694	00 757	00 820	00 883	00 945	32	01 008	01 070	01 133	01 195	01 255
33	00 552	00 615	00 678	00 741	00 803	33	00 866	00 928	00 991	01 053	01 113
34	00 411	00 474	00 537	00 600	00 662	34	00 725	00 787	00 850	00 912	00 972
35	00 270	00 333	00 396	00 459	00 521	35	00 584	00 646	00 709	00 771	00 831
36	00 130	00 193	00 256	00 319	00 381	36	00 444	00 506	00 569	00 631	00 691

---

* The logarithms up to and including 24° C. were taken from Küster-Thiel, "Logarithmische Rechentafeln für Chemiker," Berlin and Leipzig, Walter de Gruyter & Co., 1935, and the logarithms from 25° up to and including 36° C. were interpolated.

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	$p=700$	701	702	703	704	$t°$	$p=705$	706	707	708	709
10	04 571	04 633	04 695	04 757	04 819	10	04 880	04 942	05 003	05 065	05 126
11	04 418	04 480	04 542	04 604	04 666	11	04 727	04 789	04 850	04 912	04 973
12	04 265	04 327	04 389	04 451	04 513	12	04 574	04 636	04 697	04 759	04 820
13.	04 113	04 175	04 237	04 299	04 361	13	04 422	04 484	04 545	04 607	04 668
14	03 961	04 023	04 085	04 147	04 209	14	04 270	04 332	04 393	04 455	04 516
15	03 809	03 871	03 933	03 995	04 057	15	04 118	04 180	04 241	04 303	04 364
16	03 658	03 720	03 782	03 844	03 906	16	03 967	04 029	04 090	04 152	04 213
17	03 508	03 570	03 632	03 694	03 756	17	03 817	03 879	03 940	04 002	04 063
18	03 358	03 420	03 482	03 544	03 606	18	03 667	03 729	03 790	03 852	03 913
19	03 209	03 271	03 333	03 395	03 457	19	03 518	03 580	03 641	03 703	03 764
20	03 060	03 122	03 184	03 246	03 308	20	03 369	03 431	03 492	03 554	03 615
21	02 912	02 974	03 036	03 098	03 160	21	03 221	03 283	03 344	03 406	03 467
22	02 764	02 826	02 888	02 950	03 012	22	03 073	03 135	03 196	03 258	03 319
23	02 617	02 679	02 741	02 803	02 865	23	02 926	02 988	03 049	03 111	03 172
24	02 471	02 533	02 595	02 657	02 719	24	02 780	02 842	02 903	02 965	03 026
25	02 325	02 387	02 449	02 511	02 573	25	02 634	02 696	02 757	02 819	02 880
26	02 180	02 242	02 304	02 366	02 428	26	02 489	02 551	02 612	02 674	02 735
27	02 035	02 097	02 159	02 221	02 283	27	02 344	02 406	02 467	02 529	02 590
28	01 891	01 953	02 015	02 077	02 139	28	02 200	02 262	02 323	02 385	02 446
29	01 747	01 809	01 871	01 933	01 995	29	02 056	02 118	02 179	02 241	02 302
30	01 604	01 666	01 728	01 790	01 852	30	01 913	01 975	02 036	02 098	02 159
31	01 461	01 523	01 585	01 647	01 709	31	01 770	01 832	01 893	01 955	02 016
32	01 319	01 381	01 443	01 505	01 567	32	01 628	01 690	01 751	01 813	01 874
33	01 177	01 239	01 301	01 363	01 425	33	01 486	01 548	01 609	01 671	01 732
34	01 036	01 098	01 160	01 222	01 284	34	01 345	01 407	01 468	01 530	01 591
35	00 895	00 957	01 019	01 081	01 143	35	01 205	01 267	01 327	01 389	01 450
36	00 755	00 817	00 879	00 941	01 003	36	01 065	01 127	01 187	01 249	01 310

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at *t*° and *p* mm.

t°	*p*=710	711	712	713	714	*t*°	*p*=715	716	717	718	719
10	05 189	05 250	05 311	05 372	05 433	10	05 494	05 554	05 615	05 675	05 736
11	05 035	05 096	05 157	05 218	05 279	11	05 340	05 400	05 461	05 521	05 582
12	04 882	04 943	05 004	05 065	05 126	12	05 187	05 247	05 308	05 368	05 429
13	04 730	04 791	04 852	04 913	04 974	13	05 035	05 095	05 156	05 216	05 277
14	04 578	04 639	04 700	04 761	04 822	14	04 883	04 943	05 004	05 064	05 125
15	04 427	04 488	04 549	04 610	04 671	15	04 732	04 792	04 853	04 913	04 974
16	04 276	04 337	04 398	04 459	04 520	16	04 581	04 641	04 702	04 762	04 823
17	04 126	04 187	04 248	04 309	04 370	17	04 431	04 491	04 552	04 612	04 673
18	03 976	04 037	04 098	04 159	04 220	18	04 281	04 341	04 402	04 462	04 523
19	03 827	03 888	03 949	04 010	04 071	19	04 132	04 192	04 253	04 313	04 374
20	03 678	03 739	03 800	03 861	03 922	20	03 983	04 043	04 104	04 164	04 225
21	03 530	03 591	03 652	03 713	03 774	21	03 835	03 895	03 956	04 016	04 077
22	03 382	03 443	03 504	03 565	03 626	22	03 687	03 747	03 808	03 868	03 929
23	03 235	03 296	03 357	03 418	03 479	23	03 540	03 600	03 661	03 721	03 782
24	03 088	03 149	03 210	03 271	03 332	24	03 393	03 453	03 514	03 574	03 635
25	02 942	03 003	03 064	03 125	03 186	25	03 247	03 307	03 368	03 428	03 489
26	02 797	02 858	02 919	02 980	03 041	26	03 102	03 162	03 223	03 283	03 344
27	02 652	02 713	02 774	02 835	02 896	27	02 957	03 017	03 078	03 138	03 199
28	02 508	02 569	02 630	02 691	02 752	28	02 813	02 873	02 934	02 994	03 055
29	02 364	02 425	02 486	02 547	02 603	29	02 669	02 729	02 790	02 850	02 911
30	02 221	02 282	02 343	02 404	02 465	30	02 526	02 586	02 647	02 707	02 768
31	02 078	02 139	02 203	02 261	02 322	31	02 383	02 443	02 504	02 564	02 625
32	01 936	01 997	02 061	02 119	02 180	32	02 241	02 301	02 362	02 422	02 483
33	01 794	01 855	01 919	01 977	02 038	33	02 099	02 159	02 220	02 280	02 341
34	01 653	01 714	01 778	01 836	01 897	34	01 958	02 018	02 079	02 139	02 200
35	01 512	01 573	01 637	01 695	01 756	35	01 817	01 877	01 938	01 998	02 059
36	01 372	01 433	01 497	01 555	01 616	36	01 677	01 737	01 798	01 858	01 919

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	$p=720$	721	722	723	724	$t°$	$p=725$	726	727	728	729
10	05 796	05 857	05 917	05 977	06 037	10	06 097	06 157	06 216	06 276	06 336
11	05 642	05 703	05 763	05 823	05 883	11	05 943	06 003	06 062	06 122	06 182
12	05 489	05 550	05 610	05 670	05 730	12	05 790	05 850	05 909	05 969	06 029
13	05 337	05 398	05 458	05 518	05 578	13	05 638	05 698	05 757	05 817	05 877
14	05 185	05 246	05 306	05 366	05 426	14	05 486	05 546	05 605	05 665	05 725
15	05 034	05 095	05 155	05 215	05 275	15	05 335	05 395	05 454	05 514	05 574
16	04 883	04 944	05 004	05 064	05 124	16	05 184	05 244	05 303	05 363	05 423
17	04 733	04 794	04 854	04 914	04 974	17	05 034	05 094	05 153	05 213	05 273
18	04 583	04 644	04 704	04 764	04 824	18	04 884	04 944	05 003	05 063	05 123
19	04 434	04 495	04 555	04 615	04 675	19	04 735	04 795	04 854	04 914	04 974
20	04 285	04 346	04 406	04 466	04 526	20	04 586	04 646	04 705	04 765	04 825
21	04 137	04 198	04 258	04 318	04 378	21	04 438	04 498	04 557	04 617	04 677
22	03 989	04 050	04 110	04 170	04 230	22	04 290	04 350	04 409	04 469	04 529
23	03 842	03 903	03 963	04 023	04 083	23	04 143	04 203	04 262	04 322	04 382
24	03 695	03 756	03 816	03 876	03 936	24	03 996	04 056	04 115	04 175	04 235
25	03 549	03 610	03 670	03 730	03 790	25	03 850	03 910	03 969	04 029	04 089
26	03 404	03 465	03 525	03 585	03 645	26	03 705	03 765	03 824	03 884	03 944
27	03 259	03 320	03 380	03 440	03 500	27	03 560	03 620	03 679	03 739	03 799
28	03 115	03 176	03 236	03 296	03 356	28	03 416	03 476	03 535	03 595	03 655
29	02 971	03 032	03 092	03 152	03 212	29	03 272	03 332	03 391	03 451	03 511
30	02 828	02 889	02 949	03 009	03 069	30	03 129	03 189	03 248	03 308	03 368
31	02 685	02 746	02 806	02 866	02 926	31	02 986	03 046	03 105	03 165	03 225
32	02 543	02 604	02 664	02 724	02 784	32	02 844	02 904	02 963	03 023	03 083
33	02 401	02 462	02 522	02 582	02 642	33	02 702	02 762	02 821	02 881	02 941
34	02 260	02 321	02 381	02 441	02 501	34	02 561	02 621	02 680	02 740	02 800
35	02 119	02 180	02 240	02 300	02 360	35	02 420	02 480	02 539	02 599	02 659
36	01 979	02 040	02 100	02 160	02 220	36	02 280	02 340	02 399	02 459	02 519

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	$p = 730$	731	732	733	734	$t°$	$p = 735$	736	737	738	739
10	06 395	06 455	06 514	06 573	06 633	10	06 692	06 751	06 810	06 869	06 927
11	06 241	06 301	06 360	06 419	06 479	11	06 538	06 597	06 656	06 715	06 773
12	06 088	06 148	06 207	06 266	06 326	12	06 385	06 444	06 503	06 562	06 620
13	05 936	05 996	06 055	06 114	06 174	13	06 233	06 292	06 351	06 410	06 468
14	05 784	05 844	05 903	05 962	06 022	14	06 081	06 140	06 199	06 258	06 316
15	05 633	05 693	05 752	05 811	05 871	15	05 930	05 989	06 048	06 107	06 165
16	05 482	05 542	05 601	05 660	05 720	16	05 779	05 838	05 897	05 956	06 014
17	05 332	05 392	05 451	05 510	05 570	17	05 629	05 688	05 747	05 806	05 864
18	05 182	05 242	05 301	05 360	05 420	18	05 479	05 538	05 597	05 656	05 714
19	05 033	05 093	05 152	05 211	05 271	19	05 330	05 389	05 448	05 507	05 565
20	04 884	04 944	05 003	05 062	05 122	20	05 181	05 240	05 299	05 358	05 416
21	04 736	04 796	04 855	04 914	04 974	21	05 033	05 092	05 151	05 210	05 268
22	04 588	04 648	04 707	04 766	04 826	22	04 885	04 944	05 003	05 062	05 120
23	04 441	04 501	04 560	04 619	04 679	23	04 738	04 797	04 856	04 915	04 973
24	04 294	04 354	04 413	04 472	04 532	24	04 591	04 650	04 709	04 768	04 826
25	04 148	04 208	04 267	04 326	04 386	25	04 445	04 504	04 563	04 622	04 680
26	04 003	04 063	04 122	04 181	04 241	26	04 300	04 359	04 418	04 477	04 535
27	03 858	03 918	03 977	04 036	04 096	27	04 155	04 214	04 273	04 332	04 390
28	03 714	03 774	03 833	03 892	03 952	28	04 011	04 070	04 129	04 188	04 246
29	03 570	03 630	03 689	03 748	03 808	29	03 867	03 926	03 985	04 044	04 102
30	03 427	03 487	03 546	03 605	03 665	30	03 724	03 783	03 842	03 901	03 959
31	03 284	03 344	03 403	03 462	03 522	31	03 581	03 640	03 699	03 758	03 816
32	03 142	03 202	03 261	03 320	03 380	32	03 439	03 498	03 557	03 616	03 674
33	03 000	03 060	03 119	03 178	03 238	33	03 297	03 356	03 415	03 474	03 532
34	02 859	02 919	02 978	03 037	03 097	34	03 156	03 215	03 274	03 333	03 391
35	02 718	02 778	02 837	02 896	02 956	35	03 015	03 074	03 133	03 192	03 250
36	02 578	02 638	02 697	02 756	02 816	36	02 875	02 934	02 993	03 052	03 110

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	$p = 740$	741	742	743	744	$t°$	$p = 745$	746	747	748	749
10	06 986	07 045	07 103	07 162	07 220	10	07 279	07 337	07 395	07 453	07 511
11	06 832	06 891	06 949	07 008	07 066	11	07 125	07 183	07 241	07 299	07 357
12	06 679	06 738	06 796	06 855	06 913	12	06 972	07 030	07 088	07 146	07 204
13	06 527	06 586	06 644	06 703	06 761	13	06 820	06 878	06 936	06 994	07 052
14	06 375	06 434	06 492	06 551	06 609	14	06 668	06 726	06 784	06 842	06 900
15	06 224	06 283	06 341	06 400	06 458	15	06 517	06 575	06 633	06 691	06 749
16	06 073	06 132	06 190	06 249	06 307	16	06 366	06 424	06 482	06 540	06 598
17	05 923	05 982	06 040	06 099	06 157	17	06 216	06 274	06 332	06 390	06 448
18	05 773	05 832	05 890	05 949	06 007	18	06 066	06 124	06 182	06 240	06 298
19	05 624	05 683	05 741	05 800	05 858	19	05 917	05 975	06 033	06 091	06 149
20	05 475	05 534	05 592	05 651	05 709	20	05 768	05 826	05 884	05 942	06 000
21	05 327	05 386	05 444	05 503	05 561	21	05 620	05 678	05 736	05 794	05 852
22	05 179	05 238	05 296	05 355	05 413	22	05 472	05 530	05 588	05 646	05 704
23	05 032	05 091	05 149	05 208	05 266	23	05 325	05 383	05 441	05 499	05 557
24	04 885	04 944	05 002	05 061	05 119	24	05 178	05 236	05 294	05 352	05 410
25	04 739	04 798	04 856	04 915	04 973	25	05 032	05 090	05 148	05 206	05 264
26	04 594	04 653	04 711	04 770	04 828	26	04 887	04 945	05 003	05 061	05 119
27	04 449	04 508	04 566	04 625	04 683	27	04 742	04 800	04 858	04 916	04 974
28	04 305	04 364	04 422	04 481	04 539	28	04 598	04 656	04 714	04 772	04 830
29	04 161	04 220	04 278	04 337	04 395	29	04 454	04 512	04 570	04 628	04 686
30	04 018	04 078	04 135	04 194	04 252	30	04 311	04 369	04 428	04 485	04 543
31	03 875	03 935	03 992	04 051	04 109	31	04 168	04 226	04 285	04 342	04 400
32	03 733	03 793	03 850	03 909	03 967	32	04 026	04 084	04 143	04 200	04 258
33	03 591	03 651	03 708	03 767	03 825	33	03 884	03 942	04 001	04 058	04 116
34	03 450	03 510	03 567	03 626	03 684	34	03 743	03 801	03 860	03 917	03 975
35	03 309	03 369	03 426	03 485	03 543	35	03 602	03 660	03 719	03 776	03 834
36	03 169	03 229	03 286	03 345	03 403	36	03 462	03 520	03 579	03 636	03 694

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	$p=750$	751	752	753	754	$t°$	$p=755$	756	757	758	759
10	07 569	07 627	07 685	07 742	07 800	10	07 858	07 915	07 973	08 030	08 087
11	07 415	07 473	07 531	07 588	07 646	11	07 704	07 761	07 819	07 876	07 933
12	07 262	07 320	07 378	07 435	07 493	12	07 551	07 608	07 666	07 723	07 780
13	07 110	07 168	07 226	07 283	07 341	13	07 399	07 456	07 514	07 571	07 628
14	06 958	07 016	07 074	07 131	07 189	14	07 247	07 304	07 362	07 419	07 476
15	06 807	06 865	06 923	06 980	07 038	15	07 096	07 153	07 211	07 268	07 325
16	06 656	06 714	06 772	06 829	06 887	16	06 945	07 002	07 060	07 117	07 174
17	06 506	06 564	06 622	06 679	06 737	17	06 795	06 852	06 910	06 967	07 024
18	06 356	06 414	06 472	06 529	06 587	18	06 645	06 702	06 760	06 817	06 874
19	06 207	06 265	06 323	06 380	06 438	19	06 496	06 553	06 611	06 668	06 725
20	06 058	06 116	06 174	06 231	06 289	20	06 347	06 404	06 462	06 519	06 576
21	05 910	05 968	06 026	06 083	06 141	21	06 199	06 256	06 314	06 371	06 428
22	05 762	05 820	05 878	05 935	05 993	22	06 051	06 108	06 166	06 223	06 280
23	05 615	05 673	05 731	05 788	05 846	23	05 904	05 961	06 019	06 076	06 133
24	05 468	05 526	05 584	05 641	05 699	24	05 757	05 814	05 872	05 929	05 986
25	05 322	05 380	05 438	05 495	05 553	25	05 611	05 668	05 726	05 783	05 840
26	05 177	05 235	05 293	05 350	05 408	26	05 466	05 523	05 581	05 638	05 695
27	05 032	05 090	05 148	05 205	05 263	27	05 321	05 378	05 436	05 493	05 550
28	04 888	04 946	05 004	05 061	05 119	28	05 177	05 234	05 292	05 349	05 406
29	04 744	04 802	04 860	04 917	04 975	29	05 033	05 090	05 148	05 205	05 262
30	04 601	04 659	04 717	04 774	04 832	30	04 890	04 947	05 005	05 062	05 119
31	04 458	04 516	04 574	04 631	04 689	31	04 747	04 804	04 862	04 919	04 976
32	04 316	04 374	04 432	04 489	04 547	32	04 605	04 662	04 720	04 777	04 834
33	04 174	04 232	04 290	04 347	04 405	33	04 463	04 520	04 578	04 635	04 692
34	04 033	04 091	04 149	04 206	04 264	34	04 322	04 379	04 437	04 494	04 551
35	03 892	03 950	04 008	04 065	04 123	35	04 181	04 238	04 296	04 353	04 410
36	03 752	03 810	03 868	03 925	03 983	36	04 041	04 098	04 156	04 213	04 270

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	$p=760$	761	762	763	764	$t°$	$p=765$	766	767	768	769
10	08 144	08 201	08 258	08 315	08 372	10	08 429	08 486	08 543	08 599	08 656
11	07 990	08 047	08 104	08 161	08 218	11	08 275	08 332	08 389	08 445	08 502
12	07 837	07 894	07 951	08 008	08 065	12	08 122	08 179	08 236	08 292	08 349
13	07 685	07 742	07 799	07 856	07 913	13	07 970	08 027	08 084	08 140	08 197
14	07 533	07 590	07 647	07 704	07 761	14	07 818	07 875	07 932	07 988	08 045
15	07 382	07 439	07 496	07 553	07 610	15	07 667	07 724	07 781	07 837	07 894
16	07 231	07 288	07 345	07 402	07 459	16	07 516	07 573	07 630	07 686	07 743
17	07 081	07 138	07 195	07 252	07 309	17	07 366	07 423	07 480	07 536	07 593
18	06 931	06 988	07 045	07 102	07 159	18	07 216	07 273	07 330	07 386	07 443
19	06 782	06 839	06 896	06 953	07 010	19	07 067	07 124	07 181	07 237	07 294
20	06 633	06 690	06 747	06 804	06 861	20	06 918	06 975	07 032	07 088	07 145
21	06 485	06 542	06 599	06 656	06 713	21	06 770	06 827	06 884	06 940	06 997
22	06 337	06 394	06 451	06 508	06 565	22	06 622	06 679	06 736	06 792	06 849
23	06 190	06 247	06 304	06 361	06 418	23	06 475	06 532	06 589	06 645	06 702
24	06 043	06 100	06 157	06 214	06 271	24	06 328	06 385	06 442	06 498	06 555
25	05 897	05 954	06 011	06 068	06 125	25	06 182	06 239	06 296	06 352	06 409
26	05 752	05 809	05 866	05 923	05 980	26	06 037	06 094	06 151	06 207	06 264
27	05 607	05 664	05 721	05 778	05 835	27	05 892	05 949	06 006	06 062	06 119
28	05 463	05 520	05 577	05 634	05 691	28	05 748	05 805	05 862	05 918	05 975
29	05 319	05 376	05 433	05 490	05 547	29	05 604	05 661	05 718	05 774	05 831
30	05 176	05 233	05 290	05 347	05 404	30	05 461	05 518	05 575	05 631	05 688
31	05 033	05 090	05 147	05 204	05 261	31	05 318	05 375	05 432	05 488	05 545
32	04 891	04 948	05 005	05 062	05 119	32	05 176	05 233	05 290	05 346	05 403
33	04 749	04 806	04 863	04 920	04 977	33	05 034	05 091	05 148	05 204	05 261
34	04 608	04 665	04 722	04 779	04 836	34	04 893	04 950	05 007	05 063	05 120
35	04 467	04 524	04 581	04 638	04 695	35	04 752	04 809	04 866	04 922	04 979
36	04 327	04 384	04 441	04 498	04 555	36	04 612	04 669	04 726	04 782	04 839

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at $t°$ and p mm.

$t°$	p = 770	771	772	773	774	$t°$	p = 775	776	777	778	779
10	08 712	08 768	08 825	08 881	08 937	10	08 993	09 049	09 105	09 161	09 217
11	08 558	08 614	08 671	08 727	08 783	11	08 839	08 895	08 951	09 007	09 063
12	08 405	08 461	08 518	08 574	08 630	12	08 686	08 742	08 798	08 854	08 910
13	08 253	08 309	08 366	08 422	08 478	13	08 534	08 590	08 646	08 702	08 758
14	08 101	08 157	08 214	08 270	08 326	14	08 382	08 438	08 494	08 550	08 606
15	07 949	08 005	08 062	08 118	08 174	15	08 230	08 286	08 342	08 398	08 454
16	07 798	07 854	07 911	07 967	08 023	16	08 079	08 135	08 191	08 247	08 303
17	07 648	07 704	07 761	07 817	07 873	17	07 929	07 985	08 041	08 097	08 153
18	07 498	07 554	07 611	07 667	07 723	18	07 779	07 835	07 891	07 947	08 003
19	07 349	07 405	07 462	07 518	07 574	19	07 630	07 686	07 742	07 798	07 854
20	07 200	07 256	07 313	07 369	07 425	20	07 481	07 537	07 593	07 649	07 705
21	07 052	07 108	07 165	07 221	07 277	21	07 333	07 389	07 445	07 501	07 557
22	06 904	06 960	07 017	07 073	07 129	22	07 185	07 241	07 297	07 353	07 409
23	06 757	06 813	06 870	06 926	06 982	23	07 038	07 094	07 150	07 206	07 262
24	06 611	06 667	06 724	06 780	06 836	24	06 892	06 948	07 004	07 060	07 116
25	06 464	06 523	06 578	06 634	06 690	25	06 746	06 802	06 858	06 914	06 970
26	06 320	06 378	06 433	06 489	06 545	26	06 601	06 657	06 713	06 769	06 825
27	06 175	06 233	06 288	06 344	06 400	27	06 456	06 512	06 568	06 624	06 680
28	06 031	06 089	06 144	06 200	06 256	28	06 312	06 368	06 424	06 480	06 536
29	05 887	05 945	06 000	06 056	06 112	29	06 168	06 224	06 280	06 336	06 392
30	05 744	05 802	05 857	05 913	05 969	30	06 025	06 081	06 137	06 193	06 249
31	05 601	05 659	05 714	05 770	05 826	31	05 882	05 938	05 994	06 050	06 106
32	05 459	05 517	05 573	05 628	05 684	32	05 740	05 796	05 852	05 908	05 964
33	05 317	05 375	05 431	05 486	05 542	33	05 598	05 654	05 710	05 756	05 822
34	05 176	05 234	05 290	05 345	05 401	34	05 457	05 513	05 569	05 615	05 681
35	05 035	05 093	05 149	05 204	05 260	35	05 316	05 372	05 428	05 474	05 540
36	04 895	04 953	05 009	05 064	05 120	36	05 176	05 232	05 288	05 334	05 400

NITROGEN REDUCTION TABLE—*Continued*

One milliliter of nitrogen at 0° and 760 mm. pressure weighs 1.2505 mg. This table gives the logarithm of the weight of 1 ml. of nitrogen at t° and p mm.

t°	$p = 780$	781	782	783	784	t°	$p = 785$	786	787	788	789
10	09 272	09 328	09 384	09 439	09 495	10	09 550	09 605	09 660	09 716	09 771
11	09 118	09 174	09 230	09 285	09 341	11	09 396	09 451	09 506	09 562	09 617
12	08 965	09 021	09 077	09 132	09 188	12	09 243	09 298	09 353	09 409	09 464
13	08 813	08 869	08 925	08 980	09 036	13	09 091	09 146	09 201	09 257	09 312
14	08 661	08 717	08 773	08 828	08 884	14	08 939	08 994	09 049	09 105	09 162
15	08 509	08 565	08 621	08 676	08 732	15	08 787	08 842	08 897	08 953	09 008
16	08 358	08 414	08 470	08 525	08 581	16	08 636	08 691	08 746	08 802	08 857
17	08 208	08 264	08 320	08 375	08 431	17	08 486	08 541	08 596	08 652	08 707
18	08 058	08 114	08 170	08 225	08 281	18	08 336	08 391	08 446	08 502	08 557
19	07 909	07 965	08 021	08 076	08 132	19	08 187	08 242	08 297	08 353	08 408
20	07 760	07 816	07 872	07 927	07 983	20	08 038	08 093	08 148	08 204	08 259
21	07 612	07 668	07 724	07 779	07 835	21	07 890	07 945	08 000	08 056	08 111
22	07 464	07 520	07 576	07 631	07 687	22	07 742	07 797	07 852	07 908	07 963
23	07 317	07 373	07 429	07 484	07 540	23	07 595	07 650	07 705	07 761	07 816
24	07 171	07 227	07 283	07 338	07 394	24	07 449	07 504	07 559	07 615	07 670
25	07 025	07 081	07 137	07 192	07 248	25	07 303	07 358	07 413	07 469	07 524
26	06 880	06 936	06 992	07 047	07 103	26	07 158	07 213	07 268	07 324	07 379
27	06 735	06 771	06 847	06 902	06 958	27	07 013	07 068	07 123	07 179	07 234
28	06 591	06 627	06 703	06 758	06 814	28	06 869	06 924	06 979	07 035	07 090
29	06 447	06 483	06 559	06 614	06 670	29	06 725	06 780	06 835	06 891	06 946
30	06 304	06 340	06 416	06 471	06 527	30	06 582	06 637	06 692	06 748	06 803
31	06 161	06 197	06 273	06 328	06 387	31	06 439	06 494	06 549	06 605	06 660
32	06 019	06 055	06 131	06 186	06 245	32	06 297	06 352	06 407	06 463	06 518
33	05 877	05 913	05 989	06 044	06 103	33	06 155	06 210	06 265	06 321	06 376
34	05 736	05 772	05 848	05 903	05 962	34	06 014	06 069	06 124	06 180	06 235
35	05 595	05 631	05 707	05 762	05 821	35	05 873	05 928	05 983	06 039	06 094
36	05 455	05 491	05 567	05 622	05 681	36	05 733	05 788	05 843	05 899	05 954

N	0	1	2	3	4	5	6	7	8	9
100	00 000	043	087	130	173	217	260	303	346	389
01	432	475	518	561	604	647	689	732	775	817
02	00 860	903	945	988	*030	*072	*115	*157	*199	*242
03	01 284	326	368	410	452	494	536	578	620	662
04	01 703	745	787	828	870	912	953	995	*036	*078
05	02 119	160	202	243	284	325	366	407	449	490
06	531	572	612	653	694	735	776	816	857	898
07	02 938	979	*019	*060	*100	*141	*181	*222	*262	*302
08	03 342	383	423	463	503	543	583	623	663	703
09	03 743	782	822	862	902	941	981	*021	*060	*100
110	04 139	179	218	258	297	336	376	415	454	493
11	532	571	610	650	689	727	766	805	844	883
12	04 922	961	999	*038	*077	*115	*154	*192	*231	*269
13	05 308	346	385	423	461	500	538	576	614	652
14	05 690	729	767	805	843	881	918	956	994	*032
15	06 070	108	145	183	221	258	296	333	371	408
16	446	483	521	558	595	633	670	707	744	781
17	06 819	856	893	930	967	*004	*041	*078	*115	*151
18	07 188	225	262	298	335	372	408	445	482	518
19	555	591	628	664	700	737	773	809	846	882
120	07 918	954	990	*027	*063	*099	*135	*171	*207	*243
21	08 279	314	350	386	422	458	493	529	565	600
22	636	672	707	743	778	814	849	884	920	955
23	08 991	*026	*061	*096	*132	*167	*202	*237	*272	*307
24	09 342	377	412	447	482	517	552	587	621	656
25	09 691	726	760	795	830	864	899	934	968	*003
26	10 037	072	106	140	175	209	243	278	312	346
27	380	415	449	483	517	551	585	619	653	687
28	10 721	755	789	823	857	890	924	958	992	*025
29	11 059	093	126	160	193	227	261	294	327	361
130	394	428	461	494	528	561	594	628	661	694
31	11 727	760	793	826	860	893	926	959	992	*024
32	12 057	090	123	156	189	222	254	287	320	352
33	385	418	450	483	516	548	581	613	646	678
34	12 710	743	775	808	840	872	905	937	969	*001
35	13 033	066	098	130	162	194	226	258	290	322
36	354	386	418	450	481	513	545	577	609	640
37	672	704	735	767	799	830	862	893	925	956
38	13 988	*019	*051	*082	*114	*145	*176	*208	*239	*270
39	14 301	333	364	395	426	457	489	520	551	582
140	613	644	675	706	737	768	799	829	860	891
41	14 922	953	983	*014	*045	*076	*106	*137	*168	*198
42	15 229	259	290	320	351	381	412	442	473	503
43	534	564	594	625	655	685	715	746	776	806
44	15 836	866	897	927	957	987	*017	*047	*077	*107
45	16 137	167	197	227	256	286	316	346	376	406
46	435	465	495	524	554	584	613	643	673	702
47	16 732	761	791	820	850	879	909	938	967	997
48	17 026	056	085	114	143	173	202	231	260	289
49	319	348	377	406	435	464	493	522	551	580
150	17 609	638	667	696	725	754	782	811	840	869
N	0	1	2	3	4	5	6	7	8	9

Prop. Parts

	44	**43**	**42**
1	4.4	4.3	4.2
2	8.8	8.6	8.4
3	13.2	12.9	12.6
4	17.6	17.2	16.8
5	22.0	21.5	21.0
6	26.4	25.8	25.2
7	30.8	30.1	29.4
8	35.2	34.4	33.6
9	39.6	38.7	37.8

	41	**40**	**39**
1	4.1	4	3.9
2	8.2	8	7.8
3	12.3	12	11.7
4	16.4	16	15.6
5	20.5	20	19.5
6	24.6	24	23.4
7	28.7	28	27.3
8	32.8	32	31.2
9	36.9	36	35.1

	38	**37**	**36**
1	3.8	3.7	3.6
2	7.6	7.4	7.2
3	11.4	11.1	10.8
4	15.2	14.8	14.4
5	19.0	18.5	18.0
6	22.8	22.2	21.6
7	26.6	25.9	25.2
8	30.4	29.6	28.8
9	34.2	33.3	32.4

	35	**34**	**33**
1	3.5	3.4	3.3
2	7.0	6.8	6.6
3	10.5	10.2	9.9
4	14.0	13.6	13.2
5	17.5	17.0	16.5
6	21.0	20.4	19.8
7	24.5	23.8	23.1
8	28.0	27.2	26.4
9	31.5	30.6	29.7

	32	**31**	**30**
1	3.2	3.1	3
2	6.4	6.2	6
3	9.6	9.3	9
4	12.8	12.4	12
5	16.0	15.5	15
6	19.2	18.6	18
7	22.4	21.7	21
8	25.6	24.8	24
9	28.8	27.9	27

Prop. Parts

Prop. Parts

	29	28
1	2.9	2.8
2	5.8	5.6
3	8.7	8.4
4	11.6	11.2
5	14.5	14.0
6	17.4	16.8
7	20.3	19.6
8	23.2	22.4
9	26.1	25.2

	27	26
1	2.7	2.6
2	5.4	5.2
3	8.1	7.8
4	10.8	10.4
5	13.5	13.0
6	16.2	15.6
7	18.9	18.2
8	21.6	20.8
9	24.3	23.4

	25
1	2.5
2	5.0
3	7.5
4	10.0
5	12.5
6	15.0
7	17.5
8	20.0
9	22.5

	24	23
1	2.4	2.3
2	4.8	4.6
3	7.2	6.9
4	9.6	9.2
5	12.0	11.5
6	14.4	13.8
7	16.8	16.1
8	19.2	18.4
9	21.6	20.7

	22	21
1	2.2	2.1
2	4.4	4.2
3	6.6	6.3
4	8.8	8.4
5	11.0	10.5
6	13.2	12.6
7	15.4	14.7
8	17.6	16.8
9	19.8	18.9

N	0	1	2	3	4	5	6	7	8	9
150	17 609	638	667	696	725	754	782	811	840	869
51	17 898	926	955	984	*013	*041	*070	*099	*127	*156
52	18 184	213	241	270	298	327	355	384	412	441
53	469	498	526	554	583	611	639	667	696	724
54	18 752	780	808	837	865	893	921	949	977	*005
55	19 033	061	089	117	145	173	201	229	257	285
56	312	340	368	396	424	451	479	507	535	562
57	590	618	645	673	700	728	756	783	811	838
58	19 866	893	921	948	976	*003	*030	*058	*085	*112
59	20 140	167	194	222	249	276	303	330	358	385
160	412	439	466	493	520	548	575	602	629	656
61	683	710	737	763	790	817	844	871	898	925
62	20 952	978	*005	*032	*059	*085	*112	*139	*165	*192
63	21 219	245	272	299	325	352	378	405	431	458
64	484	511	537	564	590	617	643	669	696	722
65	21 748	775	801	827	854	880	906	932	958	985
66	22 011	037	063	089	115	141	167	194	220	246
67	272	298	324	350	376	401	427	453	479	505
68	531	557	583	608	634	660	686	712	737	763
69	22 789	814	840	866	891	917	943	968	994	*019
170	23 045	070	096	121	147	172	198	223	249	274
71	300	325	350	376	401	426	452	477	502	528
72	553	578	603	629	654	679	704	729	754	779
73	23 805	830	855	880	905	930	955	980	*005	*030
74	24 055	080	105	130	155	180	204	229	254	279
75	304	329	353	378	403	428	452	477	502	527
76	551	576	601	625	650	674	699	724	748	773
77	24 797	822	846	871	895	920	944	969	993	*018
78	25 042	066	091	115	139	164	188	212	237	261
79	285	310	334	358	382	406	431	455	479	503
180	527	551	575	600	624	648	672	696	720	744
81	25 768	792	816	840	864	888	912	935	959	983
82	26 007	031	055	079	102	126	150	174	198	221
83	245	269	293	316	340	364	387	411	435	458
84	482	505	529	553	576	600	623	647	670	694
85	717	741	764	788	811	834	858	881	905	928
86	26 951	975	998	*021	*045	*068	*091	*114	*138	*161
87	27 184	207	231	254	277	300	323	346	370	393
88	416	439	462	485	508	531	554	577	600	623
89	646	669	692	715	738	761	784	807	830	852
190	27 875	898	921	944	967	989	*012	*035	*058	*081
91	28 103	126	149	171	194	217	240	262	285	307
92	330	353	375	398	421	443	466	488	511	533
93	556	578	601	623	646	668	691	713	735	758
94	28 780	803	825	847	870	892	914	937	959	981
95	29 003	026	048	070	092	115	137	159	181	203
96	226	248	270	292	314	336	358	380	403	425
97	447	469	491	513	535	557	579	601	623	645
98	667	688	710	732	754	776	798	820	842	863
99	29 885	907	929	951	973	994	*016	*038	*060	*081
200	30 103	125	146	168	190	211	233	255	276	298
N	0	1	2	3	4	5	6	7	8	9

Prop. Parts

N	0	1	2	3	4	5	6	7	8	9
200	30 103	125	146	168	190	211	233	255	276	298
01	320	341	363	384	406	428	449	471	492	514
02	535	557	578	600	621	643	664	685	707	728
03	750	771	792	814	835	856	878	899	920	942
04	30 963	984	*006	*027	*048	*069	*091	*112	*133	*154
05	31 175	197	218	239	260	281	302	323	345	366
06	387	408	429	450	471	492	513	534	555	576
07	597	618	639	660	681	702	723	744	765	785
08	31 806	827	848	869	890	911	931	952	973	994
09	32 015	035	056	077	098	118	139	160	181	201
210	222	243	263	284	305	325	346	366	387	408
11	428	449	469	490	510	531	552	572	593	613
12	634	654	675	695	715	736	756	777	797	818
13	32 838	858	879	899	919	940	960	980	*001	*021
14	33 041	062	082	102	122	143	163	183	203	224
15	244	264	284	304	325	345	365	385	405	425
16	445	465	486	506	526	546	566	586	606	626
17	646	666	686	706	726	746	766	786	806	826
18	33 846	866	885	905	925	945	965	985	*005	*025
19	34 044	064	084	104	124	143	163	183	203	223
220	242	262	282	301	321	341	361	380	400	420
21	439	459	479	498	518	537	557	577	596	616
22	635	655	674	694	713	733	753	772	792	811
23	34 830	850	869	889	908	928	947	967	986	*005
24	35 025	044	064	083	102	122	141	160	180	199
25	218	238	257	276	295	315	334	353	372	392
26	411	430	449	468	488	507	526	545	564	583
27	603	622	641	660	679	698	717	736	755	774
28	793	813	832	851	870	889	908	927	946	965
29	35 984	*003	*021	*040	*059	*078	*097	*116	*135	*154
230	36 173	192	211	229	248	267	286	305	324	342
31	361	380	399	418	436	455	474	493	511	530
32	549	568	586	605	624	642	661	680	698	717
33	736	754	773	791	810	829	847	866	884	903
34	36 922	940	959	977	996	*014	*033	*051	*070	*088
35	37 107	125	144	162	181	199	218	236	254	273
36	291	310	328	346	365	383	401	420	438	457
37	475	493	511	530	548	566	585	603	621	639
38	658	676	694	712	731	749	767	785	803	822
39	37 840	858	876	894	912	931	949	967	985	*003
240	38 021	039	057	075	093	112	130	148	166	184
41	202	220	238	256	274	292	310	328	346	364
42	382	399	417	435	453	471	489	507	525	543
43	561	578	596	614	632	650	668	686	703	721
44	739	757	775	792	810	828	846	863	881	899
45	38 917	934	952	970	987	*005	*023	*041	*058	*076
46	39 094	111	129	146	164	182	199	217	235	252
47	270	287	305	322	340	358	375	393	410	428
48	445	463	480	498	515	533	550	568	585	602
49	620	637	655	672	690	707	724	742	759	777
250	39 794	811	829	846	863	881	898	915	933	950
N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

Prop. Parts

	22	**21**
1	2.2	2.1
2	4.4	4.2
3	6.6	6.3
4	8.8	8.4
5	11.0	10.5
6	13.2	12.6
7	15.4	14.7
8	17.6	16.8
9	19.8	18.9

	20
1	2
2	4
3	6
4	8
5	10
6	12
7	14
8	16
9	18

	19
1	1.9
2	3.8
3	5.7
4	7.6
5	9.5
6	11.4
7	13.3
8	15.2
9	17.1

	18
1	1.8
2	3.6
3	5.4
4	7.2
5	9.0
6	10.8
7	12.6
8	14.4
9	16.2

	17
1	1.7
2	3.4
3	5.1
4	6.8
5	8.5
6	10.2
7	11.9
8	13.6
9	15.3

Prop. Parts

Prop. Parts	N	0	1	2	3	4	5	6	7	8	9
	250	39 794	811	829	846	863	881	898	915	933	950
18 1 1.8 2 3.6 3 5.4 4 7.2 5 9.0 6 10.8 7 12.6 8 14.4 9 16.2	51	39 967	985	*002	*019	*037	*054	*071	*088	*106	*123
	52	40 140	157	175	192	209	226	243	261	278	295
	53	312	329	346	364	381	398	415	432	449	466
	54	483	500	518	535	552	569	586	603	620	637
	55	654	671	688	705	722	739	756	773	790	807
	56	824	841	858	875	892	909	926	943	960	976
	57	40 993	*010	*027	*044	*061	*078	*095	*111	*128	*145
	58	41 162	179	196	212	229	246	263	280	296	313
	59	330	347	363	380	397	414	430	447	464	481
	260	497	514	531	547	564	581	597	614	631	647
17 1 1.7 2 3.4 3 5.1 4 6.8 5 8.5 6 10.2 7 11.9 8 13.6 9 15.3	61	664	681	697	714	731	747	764	780	797	814
	62	830	847	863	880	896	913	929	946	963	979
	63	41 996	*012	*029	*045	*062	*078	*095	*111	*127	*144
	64	42 160	177	193	210	226	243	259	275	292	308
	65	325	341	357	374	390	406	423	439	455	472
	66	488	504	521	537	553	570	586	602	619	635
	67	651	667	684	700	716	732	749	765	781	797
	68	813	830	846	862	878	894	911	927	943	959
	69	42 975	991	*008	*024	*040	*056	*072	*088	*104	*120
	270	43 136	152	169	185	201	217	233	249	265	281
16 1 1.6 2 3.2 3 4.8 4 6.4 5 8.0 6 9.6 7 11.2 8 12.8 9 14.4	71	297	313	329	345	361	377	393	409	425	441
	72	457	473	489	505	521	537	553	569	584	600
	73	616	632	648	664	680	696	712	727	743	759
	74	775	791	807	823	838	854	870	886	902	917
	75	43 933	949	965	981	996	*012	*028	*044	*059	*075
	76	44 091	107	122	138	154	170	185	201	217	232
	77	248	264	279	295	311	326	342	358	373	389
	78	404	420	436	451	467	483	498	514	529	545
	79	560	576	592	607	623	638	654	669	685	700
	280	716	731	747	762	778	793	809	824	840	855
15 1 1.5 2 3.0 3 4.5 4 6.0 5 7.5 6 9.0 7 10.5 8 12.0 9 13.5	81	44 871	886	902	917	932	948	963	979	994	*010
	82	45 025	040	056	071	086	102	117	133	148	163
	83	179	194	209	225	240	255	271	286	301	317
	84	332	347	362	378	393	408	423	439	454	469
	85	484	500	515	530	545	561	576	591	606	621
	86	637	652	667	682	697	712	728	743	758	773
	87	788	803	818	834	849	864	879	894	909	924
	88	45 939	954	969	984	*000	*015	*030	*045	*060	*075
	89	46 090	105	120	135	150	165	180	195	210	225
	290	240	255	270	285	300	315	330	345	359	374
14 1 1.4 2 2.8 3 4.2 4 5.6 5 7.0 6 8.4 7 9.8 8 11.2 9 12.6	91	389	404	419	434	449	464	479	494	509	523
	92	538	553	568	583	598	613	627	642	657	672
	93	687	702	716	731	746	761	776	790	805	820
	94	835	850	864	879	894	909	923	938	953	967
	95	46 982	997	*012	*026	*041	*056	*070	*085	*100	*114
	96	47 129	144	159	173	188	202	217	232	246	261
	97	276	290	305	319	334	349	363	378	392	407
	98	422	436	451	465	480	494	509	524	538	553
	99	567	582	596	611	625	640	654	669	683	698
	300	47 712	727	741	756	770	784	799	813	828	842
Prop. Parts	**N**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

N	0	1	2	3	4	5	6	7	8	9	Prop. Parts
300	47 712	727	741	756	770	784	799	813	828	842	
01	47 857	871	885	900	914	929	943	958	972	986	
02	48 001	015	029	044	058	073	087	101	116	130	
03	144	159	173	187	202	216	230	244	259	273	**15** **1** 1.5 **2** 3.0 **3** 4.5 **4** 6.0 **5** 7.5 **6** 9.0 **7** 10.5 **8** 12.0 **9** 13.5
04	287	302	316	330	344	359	373	387	401	416	
05	430	444	458	473	487	501	515	530	544	558	
06	572	586	601	615	629	643	657	671	686	700	
07	714	728	742	756	770	785	799	813	827	841	
08	855	869	883	897	911	926	940	954	968	982	
09	48 996	*010	*024	*038	*052	*066	*080	*094	*108	*122	
310	49 136	150	164	178	192	206	220	234	248	262	
11	276	290	304	318	332	346	360	374	388	402	
12	415	429	443	457	471	485	499	513	527	541	
13	554	568	582	596	610	624	638	651	665	679	
14	693	707	721	734	748	762	776	790	803	817	
15	831	845	859	872	886	900	914	927	941	955	
16	49 969	982	996	*010	*024	*037	*051	*065	*079	*092	**14** **1** 1.4 **2** 2.8 **3** 4.2 **4** 5.6 **5** 7.0 **6** 8.4 **7** 9.8 **8** 11.2 **9** 12.6
17	50 106	120	133	147	161	174	188	202	215	229	
18	243	256	270	284	297	311	325	338	352	365	
19	379	393	406	420	433	447	461	474	488	501	
320	515	529	542	556	569	583	596	610	623	637	
21	651	664	678	691	705	718	732	745	759	772	
22	786	799	813	826	840	853	866	880	893	907	
23	50 920	934	947	961	974	987	*001	*014	*028	*041	
24	51 055	068	081	095	108	121	135	148	162	175	
25	188	202	215	228	242	255	268	282	295	308	
26	322	335	348	362	375	388	402	415	428	441	
27	455	468	481	495	508	521	534	548	561	574	
28	587	601	614	627	640	654	667	680	693	706	
29	720	733	746	759	772	786	799	812	825	838	**13** **1** 1.3 **2** 2.6 **3** 3.9 **4** 5.2 **5** 6.5 **6** 7.8 **7** 9.1 **8** 10.4 **9** 11.7
330	851	865	878	891	904	917	930	943	957	970	
31	51 983	996	*009	*022	*035	*048	*061	*075	*088	*101	
32	52 114	127	140	153	166	179	192	205	218	231	
33	244	257	270	284	297	310	323	336	349	362	
34	375	388	401	414	427	440	453	466	479	492	
35	504	517	530	543	556	569	582	595	608	621	
36	634	647	660	673	686	699	711	724	737	750	
37	763	776	789	802	815	827	840	853	866	879	
38	52 892	905	917	930	943	956	969	982	994	*007	
39	53 020	033	046	058	071	084	097	110	122	135	
340	148	161	173	186	199	212	224	237	250	263	
41	275	288	301	314	326	339	352	364	377	390	**12** **1** 1.2 **2** 2.4 **3** 3.6 **4** 4.8 **5** 6.0 **6** 7.2 **7** 8.4 **8** 9.6 **9** 10.8
42	403	415	428	441	453	466	479	491	504	517	
43	529	542	555	567	580	593	605	618	631	643	
44	656	668	681	694	706	719	732	744	757	769	
45	782	794	807	820	832	845	857	870	882	895	
46	53 908	920	933	945	958	970	983	995	*008	*020	
47	54 033	045	058	070	083	095	108	120	133	145	
48	158	170	183	195	208	220	233	245	258	270	
49	283	295	307	320	332	345	357	370	382	394	
350	54 407	419	432	444	456	469	481	494	506	518	
N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Prop. Parts**

Prop. Parts

	13
1	1.3
2	2.6
3	3.9
4	5.2
5	6.5
6	7.8
7	9.1
8	10.4
9	11.7

	12
1	1.2
2	2.4
3	3.6
4	4.8
5	6.0
6	7.2
7	8.4
8	9.6
9	10.8

	11
1	1.1
2	2.2
3	3.3
4	4.4
5	5.5
6	6.6
7	7.7
8	8.8
9	9.9

	10
1	1.0
2	2.0
3	3.0
4	4.0
5	5.0
6	6.0
7	7.0
8	8.0
9	9.0

Prop. Parts

N	0	1	2	3	4	5	6	7	8	9
350	54 407	419	432	444	456	469	481	494	506	518
51	531	543	555	568	580	593	605	617	630	642
52	654	667	679	691	704	716	728	741	753	765
53	777	790	802	814	827	839	851	864	876	888
54	54 900	913	925	937	949	962	974	986	998	*011
55	55 023	035	047	060	072	084	096	108	121	133
56	145	157	169	182	194	206	218	230	242	255
57	267	279	291	303	315	328	340	352	364	376
58	388	400	413	425	437	449	461	473	485	497
59	509	522	534	546	558	570	582	594	606	618
360	630	642	654	666	678	691	703	715	727	739
61	751	763	775	787	799	811	823	835	847	859
62	871	883	895	907	919	931	943	955	967	979
63	55 991	*003	*015	*027	*038	*050	*062	*074	*086	*098
64	56 110	122	134	146	158	170	182	194	205	217
65	229	241	253	265	277	289	301	312	324	336
66	348	360	372	384	396	407	419	431	443	455
67	467	478	490	502	514	526	538	549	561	573
68	585	597	608	620	632	644	656	667	679	691
69	703	714	726	738	750	761	773	785	797	808
370	820	832	844	855	867	879	891	902	914	926
71	56 937	949	961	972	984	996	*008	*019	*031	*043
72	57 054	066	078	089	101	113	124	136	148	159
73	171	183	194	206	217	229	241	252	264	276
74	287	299	310	322	334	345	357	368	380	392
75	403	415	426	438	449	461	473	484	496	507
76	519	530	542	553	565	576	588	600	611	623
77	634	646	657	669	680	692	703	715	726	738
78	749	761	772	784	795	807	818	830	841	852
79	864	875	887	898	910	921	933	944	955	967
380	57 978	990	*001	*013	*024	*035	*047	*058	*070	*081
81	58 092	104	115	127	138	149	161	172	184	195
82	206	218	229	240	252	263	274	286	297	309
83	320	331	343	354	365	377	388	399	410	422
84	433	444	456	467	478	490	501	512	524	535
85	546	557	569	580	591	602	614	625	636	647
86	659	670	681	692	704	715	726	737	749	760
87	771	782	794	805	816	827	838	850	861	872
88	883	894	906	917	928	939	950	961	973	984
89	58 995	*006	*017	*028	*040	*051	*062	*073	*084	*095
390	59 106	118	129	140	151	162	173	184	195	207
91	218	229	240	251	262	273	284	295	306	318
92	329	340	351	362	373	384	395	406	417	428
93	439	450	461	472	483	494	506	517	528	539
94	550	561	572	583	594	605	616	627	638	649
95	660	671	682	693	704	715	726	737	748	759
96	770	780	791	802	813	824	835	846	857	868
97	879	890	901	912	923	934	945	956	966	977
98	59 988	999	*010	*021	*032	*043	*054	*065	*076	*086
99	60 097	108	119	130	141	152	163	173	184	195
400	60 206	217	228	239	249	260	271	282	293	304
N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

N	0	1	2	3	4	5	6	7	8	9	Prop. Parts
400	60 206	217	228	239	249	260	271	282	293	304	
01	314	325	336	347	358	369	379	390	401	412	
02	423	433	444	455	466	477	487	498	509	520	
03	531	541	552	563	574	584	595	606	617	627	
04	638	649	660	670	681	692	703	713	724	735	
05	746	756	767	778	788	799	810	821	831	842	
06	853	863	874	885	895	906	917	927	938	949	
07	60 959	970	981	991	*002	*013	*023	*034	*045	*055	
08	61 066	077	087	098	109	119	130	140	151	162	
09	172	183	194	204	215	225	236	247	257	268	
410	278	289	300	310	321	331	342	352	363	374	
11	384	395	405	416	426	437	448	458	469	479	
12	490	500	511	521	532	542	553	563	574	584	
13	595	606	616	627	637	648	658	669	679	690	
14	700	711	721	731	742	752	763	773	784	794	
15	805	815	826	836	847	857	868	878	888	899	
16	61 909	920	930	941	951	962	972	982	993	*003	
17	62 014	024	034	045	055	066	076	086	097	107	
18	118	128	138	149	159	170	180	190	201	211	
19	221	232	242	252	263	273	284	294	304	315	
420	325	335	346	356	366	377	387	397	408	418	
21	428	439	449	459	469	480	490	500	511	521	
22	531	542	552	562	572	583	593	603	613	624	
23	634	644	655	665	675	685	696	706	716	726	
24	737	747	757	767	778	788	798	808	818	829	
25	839	849	859	870	880	890	900	910	921	931	
26	62 941	951	961	972	982	992	*002	*012	*022	*033	
27	63 043	053	063	073	083	094	104	114	124	134	
28	144	155	165	175	185	195	205	215	225	236	
29	246	256	266	276	286	296	306	317	327	337	
430	347	357	367	377	387	397	407	417	428	438	
31	448	458	468	478	488	498	508	518	528	538	
32	548	558	568	579	589	599	609	619	629	639	
33	649	659	669	679	689	699	709	719	729	739	
34	749	759	769	779	789	799	809	819	829	839	
35	849	859	869	879	889	899	909	919	929	939	
36	63 949	959	969	979	988	998	*008	*018	*028	*038	
37	64 048	058	068	078	088	098	108	118	128	137	
38	147	157	167	177	187	197	207	217	227	237	
39	246	256	266	276	286	296	306	316	326	335	
440	345	355	365	375	385	395	404	414	424	434	
41	444	454	464	473	483	493	503	513	523	532	
42	542	552	562	572	582	591	601	611	621	631	
43	640	650	660	670	680	689	699	709	719	729	
44	738	748	758	768	777	787	797	807	816	826	
45	836	846	856	865	875	885	895	904	914	924	
46	64 933	943	953	963	972	982	992	*002	*011	*021	
47	65 031	040	050	060	070	079	089	099	108	118	
48	128	137	147	157	167	176	186	196	205	215	
49	225	234	244	254	263	273	283	292	302	312	
450	65 321	331	341	350	360	369	379	389	398	408	
N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	Prop. Parts

	11
1	1.1
2	2.2
3	3.3
4	4.4
5	5.5
6	6.6
7	7.7
8	8.8
9	9.9

	10
1	1.0
2	2.0
3	3.0
4	4.0
5	5.0
6	6.0
7	7.0
8	8.0
9	9.0

	9
1	0.9
2	1.8
3	2.7
4	3.6
5	4.5
6	5.4
7	6.3
8	7.2
9	8.1

Prop. Parts	10
1	1.0
2	2.0
3	3.0
4	4.0
5	5.0
6	6.0
7	7.0
8	8.0
9	9.0

Prop. Parts	9
1	0.9
2	1.8
3	2.7
4	3.6
5	4.5
6	5.4
7	6.3
8	7.2
9	8.1

Prop. Parts	8
1	0.8
2	1.6
3	2.4
4	3.2
5	4.0
6	4.8
7	5.6
8	6.4
9	7.2

N	0	1	2	3	4	5	6	7	8	9
450	65 321	331	341	350	360	369	379	389	398	408
51	418	427	437	447	456	466	475	485	495	504
52	514	523	533	543	552	562	571	581	591	600
53	610	619	629	639	648	658	667	677	686	696
54	706	715	725	734	744	753	763	772	782	792
55	801	811	820	830	839	849	858	868	877	887
56	896	906	916	925	935	944	954	963	973	982
57	65 992	*001	*011	*020	*030	*039	*049	*058	*068	*077
58	66 087	096	106	115	124	134	143	153	162	172
59	181	191	200	210	219	229	238	247	257	266
460	276	285	295	304	314	323	332	342	351	361
61	370	380	389	398	408	417	427	436	445	455
62	464	474	483	492	502	511	521	530	539	549
63	558	567	577	586	596	605	614	624	633	642
64	652	661	671	680	689	699	708	717	727	736
65	745	755	764	773	783	792	801	811	820	829
66	839	848	857	867	876	885	894	904	913	922
67	66 932	941	950	960	969	978	987	997	*006	*015
68	67 025	034	043	052	062	071	080	089	099	108
69	117	127	136	145	154	164	173	182	191	201
470	210	219	228	237	247	256	265	274	284	293
71	302	311	321	330	339	348	357	367	376	385
72	394	403	413	422	431	440	449	459	468	477
73	486	495	504	514	523	532	541	550	560	569
74	578	587	596	605	614	624	633	642	651	660
75	669	679	688	697	706	715	724	733	742	752
76	761	770	779	788	797	806	815	825	834	843
77	852	861	870	879	888	897	906	916	925	934
78	67 943	952	961	970	979	988	997	*006	*015	*024
79	68 034	043	052	061	070	079	088	097	106	115
480	124	133	142	151	160	169	178	187	196	205
81	215	224	233	242	251	260	269	278	287	296
82	305	314	323	332	341	350	359	368	377	386
83	395	404	413	422	431	440	449	458	467	476
84	485	494	502	511	520	529	538	547	556	565
85	574	583	592	601	610	619	628	637	646	655
86	664	673	681	690	699	708	717	726	735	744
87	753	762	771	780	789	797	806	815	824	833
88	842	851	860	869	878	886	895	904	913	922
89	68 931	940	949	958	966	975	984	993	*002	*011
490	69 020	028	037	046	055	064	073	082	090	099
91	108	117	126	135	144	152	161	170	179	188
92	197	205	214	223	232	241	249	258	267	276
93	285	294	302	311	320	329	338	346	355	364
94	373	381	390	399	408	417	425	434	443	452
95	461	469	478	487	496	504	513	522	531	539
96	548	557	566	574	583	592	601	609	618	627
97	636	644	653	662	671	679	688	697	705	714
98	723	732	740	749	758	767	775	784	793	801
99	810	819	827	836	845	854	862	871	880	888
500	69 897	906	914	923	932	940	949	958	966	975
N	0	1	2	3	4	5	6	7	8	9

Prop. Parts

N	0	1	2	3	4	5	6	7	8	9
500	69 897	906	914	923	932	940	949	958	966	975
01	69 984	992	*001	*010	*018	*027	*036	*044	*053	*062
02	70 070	079	088	096	105	114	122	131	140	148
03	157	165	174	183	191	200	209	217	226	234
04	243	252	260	269	278	286	295	303	312	321
05	329	338	346	355	364	372	381	389	398	406
06	415	424	432	441	449	458	467	475	484	492
07	501	509	518	526	535	544	552	561	569	578
08	586	595	603	612	621	629	638	646	655	663
09	672	680	689	697	706	714	723	731	740	749
510	757	766	774	783	791	800	808	817	825	834
11	842	851	859	868	876	885	893	902	910	919
12	70 927	935	944	952	961	969	978	986	995	*003
13	71 012	020	029	037	046	054	063	071	079	088
14	096	105	113	122	130	139	147	155	164	172
15	181	189	198	206	214	223	231	240	248	257
16	265	273	282	290	299	307	315	324	332	341
17	349	357	366	374	383	391	399	408	416	425
18	433	441	450	458	466	475	483	492	500	508
19	517	525	533	542	550	559	567	575	584	592
520	600	609	617	625	634	642	650	659	667	675
21	684	692	700	709	717	725	734	742	750	759
22	767	775	784	792	800	809	817	825	834	842
23	850	858	867	875	883	892	900	908	917	925
24	71 933	941	950	958	966	975	983	991	999	*008
25	72 016	024	032	041	049	057	066	074	082	090
26	099	107	115	123	132	140	148	156	165	173
27	181	189	198	206	214	222	230	239	247	255
28	263	272	280	288	296	304	313	321	329	337
29	346	354	362	370	378	387	395	403	411	419
530	428	436	444	452	460	469	477	485	493	501
31	509	518	526	534	542	550	558	567	575	583
32	591	599	607	616	624	632	640	648	656	665
33	673	681	689	697	705	713	722	730	738	746
34	754	762	770	779	787	795	803	811	819	827
35	835	843	852	860	868	876	884	892	900	908
36	916	925	933	941	949	957	965	973	981	989
37	72 997	*006	*014	*022	*030	*038	*046	*054	*062	*070
38	73 078	086	094	102	111	119	127	135	143	151
39	159	167	175	183	191	199	207	215	223	231
540	239	247	255	263	272	280	288	296	304	312
41	320	328	336	344	352	360	368	376	384	392
42	400	408	416	424	432	440	448	456	464	472
43	480	488	496	504	512	520	528	536	544	552
44	560	568	576	584	592	600	608	616	624	632
45	640	648	656	664	672	679	687	695	703	711
46	719	727	735	743	751	759	767	775	783	791
47	799	807	815	823	830	838	846	854	862	870
48	878	886	894	902	910	918	926	933	941	949
49	73 957	965	973	981	989	997	*005	*013	*020	*028
550	74 036	044	052	060	068	076	084	092	099	107
N	0	1	2	3	4	5	6	7	8	9

Prop. Parts

	9
1	0.9
2	1.8
3	2.7
4	3.6
5	4.5
6	5.4
7	6.3
8	7.2
9	8.1

	8
1	0.8
2	1.6
3	2.4
4	3.2
5	4.0
6	4.8
7	5.6
8	6.4
9	7.2

	7
1	0.7
2	1.4
3	2.1
4	2.8
5	3.5
6	4.2
7	4.9
8	5.6
9	6.3

Prop. Parts

Prop. Parts	N	0	1	2	3	4	5	6	7	8	9
	550	74 036	044	052	060	068	076	084	092	099	107
	51	115	123	131	139	147	155	162	170	178	186
	52	194	202	210	218	225	233	241	249	257	265
	53	273	280	288	296	304	312	320	327	335	343
	54	351	359	367	374	382	390	398	406	414	421
	55	429	437	445	453	461	468	476	484	492	500
	56	507	515	523	531	539	547	554	562	570	578
	57	586	593	601	609	617	624	632	640	648	656
	58	663	671	679	687	695	702	710	718	726	733
	59	741	749	757	764	772	780	788	796	803	811
	560	819	827	834	842	850	858	865	873	881	889
	61	896	904	912	920	927	935	943	950	958	966
8	62	74 974	981	989	997	*005	*012	*020	*028	*035	*043
1 0.8 **2** 1.6	63	75 051	059	066	074	082	089	097	105	113	120
3 2.4	64	128	136	143	151	159	166	174	182	189	197
4 3.2 **5** 4.0	65	205	213	220	228	236	243	251	259	266	274
6 4.8	66	282	289	297	305	312	320	328	335	343	351
7 5.6	67	358	366	374	381	389	397	404	412	420	427
8 6.4	68	435	442	450	458	465	473	481	488	496	504
9 7.2	69	511	519	526	534	542	549	557	565	572	580
	570	587	595	603	610	618	626	633	641	648	656
	71	664	671	679	686	694	702	709	717	724	732
	72	740	747	755	762	770	778	785	793	800	808
	73	815	823	831	838	846	853	861	868	876	884
	74	891	899	906	914	921	929	937	944	952	959
	75	75 967	974	982	989	997	*005	*012	*020	*027	*035
	76	76 042	050	057	065	072	080	087	095	103	110
	77	118	125	133	140	148	155	163	170	178	185
	78	193	200	208	215	223	230	238	245	253	260
	79	268	275	283	290	298	305	313	320	328	335
	580	343	350	358	365	373	380	388	395	403	410
7	81	418	425	433	440	448	455	462	470	477	485
1 0.7	82	492	500	507	515	522	530	537	545	552	559
2 1.4 **3** 2.1	83	567	574	582	589	597	604	612	619	626	634
4 2.8	84	641	649	656	664	671	678	686	693	701	708
5 3.5	85	716	723	730	738	745	753	760	768	775	782
6 4.2	86	790	797	805	812	819	827	834	842	849	856
7 4.9 **8** 5.6	87	864	871	879	886	893	901	908	916	923	930
9 6.3	88	76 938	945	953	960	967	975	982	989	997	*004
	89	77 012	019	026	034	041	048	056	063	070	078
	590	085	093	100	107	115	122	129	137	144	151
	91	159	166	173	181	188	195	203	210	217	225
	92	232	240	247	254	262	269	276	283	291	298
	93	305	313	320	327	335	342	349	357	364	371
	94	379	386	393	401	408	415	422	430	437	444
	95	452	459	466	474	481	488	495	503	510	517
	96	525	532	539	546	554	561	568	576	583	590
	97	597	605	612	619	627	634	641	648	656	663
	98	670	677	685	692	699	706	714	721	728	735
	99	743	750	757	764	772	779	786	793	801	808
	600	77 815	822	830	837	844	851	859	866	873	880
Prop. Parts	**N**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

N	0	1	2	3	4	5	6	7	8	9	Prop. Parts
600	77 815	822	830	837	844	851	859	866	873	880	
01	887	895	902	909	916	924	931	938	945	952	
02	77 960	967	974	981	988	996	*003	*010	*017	*025	
03	78 032	039	046	053	061	068	075	082	089	097	
04	104	111	118	125	132	140	147	154	161	168	
05	176	183	190	197	204	211	219	226	233	240	
06	247	254	262	269	276	283	290	297	305	312	
07	319	326	333	340	347	355	362	369	376	383	8 **1** 0.8 **2** 1.6 **3** 2.4 **4** 3.2 **5** 4.0 **6** 4.8 **7** 5.6 **8** 6.4 **9** 7.2
08	390	398	405	412	419	426	433	440	447	455	
09	462	469	476	483	490	497	504	512	519	526	
610	533	540	547	554	561	569	576	583	590	597	
11	604	611	618	625	633	640	647	654	661	668	
12	675	682	689	696	704	711	718	725	732	739	
13	746	753	760	767	774	781	789	796	803	810	
14	817	824	831	838	845	852	859	866	873	880	
15	888	895	902	909	916	923	930	937	944	951	
16	78 958	965	972	979	986	993	*000	*007	*014	*021	
17	79 029	036	043	050	057	064	071	078	085	092	
18	099	106	113	120	127	134	141	148	155	162	
19	169	176	183	190	197	204	211	218	225	232	
620	239	246	253	260	267	274	281	288	295	302	
21	309	316	323	330	337	344	351	358	365	372	
22	379	386	393	400	407	414	421	428	435	442	7 **1** 0.7 **2** 1.4 **3** 2.1 **4** 2.8 **5** 3.5 **6** 4.2 **7** 4.9 **8** 5.6 **9** 6.3
23	449	456	463	470	477	484	491	498	505	511	
24	518	525	532	539	546	553	560	567	574	581	
25	588	595	602	609	616	623	630	637	644	650	
26	657	664	671	678	685	692	699	706	713	720	
27	727	734	741	748	754	761	768	775	782	789	
28	796	803	810	817	824	831	837	844	851	858	
29	865	872	879	886	893	900	906	913	920	927	
630	79 934	941	948	955	962	969	975	982	989	996	
31	80 003	010	017	024	030	037	044	051	058	065	
32	072	079	085	092	099	106	113	120	127	134	
33	140	147	154	161	168	175	182	188	195	202	
34	209	216	223	229	236	243	250	257	264	271	
35	277	284	291	298	305	312	318	325	332	339	
36	346	353	359	366	373	380	387	393	400	407	
37	414	421	428	434	441	448	455	462	468	475	
38	482	489	496	502	509	516	523	530	536	543	6 **1** 0.6 **2** 1.2 **3** 1.8 **4** 2.4 **5** 3.0 **6** 3.6 **7** 4.2 **8** 4.8 **9** 5.4
39	550	557	564	570	577	584	591	598	604	611	
640	618	625	632	638	645	652	659	665	672	679	
41	686	693	699	706	713	720	726	733	740	747	
42	754	760	767	774	781	787	794	801	808	814	
43	821	828	835	841	848	855	862	868	875	882	
44	889	895	902	909	916	922	929	936	943	949	
45	80 956	963	969	976	983	990	996	*003	*010	*017	
46	81 023	030	037	043	050	057	064	070	077	084	
47	090	097	104	111	117	124	131	137	144	151	
48	158	164	171	178	184	191	198	204	211	218	
49	224	231	238	245	251	258	265	271	278	285	
650	81 291	298	305	311	318	325	331	338	345	351	
N	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**Prop. Parts**

Prop. Parts	N	0	1	2	3	4	5	6	7	8	9
	650	81 291	298	305	311	318	325	331	338	345	351
	51	358	365	371	378	385	391	398	405	411	418
	52	425	431	438	445	451	458	465	471	478	485
	53	491	498	505	511	518	525	531	538	544	551
	54	558	564	571	578	584	591	598	604	611	617
	55	624	631	637	644	651	657	664	671	677	684
	56	690	697	704	710	717	723	730	737	743	750
	57	757	763	770	776	783	790	796	803	809	816
	58	823	829	836	842	849	856	862	869	875	882
	59	889	895	902	908	915	921	928	935	941	948
	660	81 954	961	968	974	981	987	994	*000	*007	*014
	61	82 020	027	033	040	046	053	060	066	073	079
	62	086	092	099	105	112	119	125	132	138	145
	63	151	158	164	171	178	184	191	197	204	210
	64	217	223	230	236	243	249	256	263	269	276
	65	282	289	295	302	308	315	321	328	334	341
	66	347	354	360	367	373	380	387	393	400	406
	67	413	419	426	432	439	445	452	458	465	471
	68	478	484	491	497	504	510	517	523	530	536
	69	543	549	556	562	569	575	582	588	595	601
	670	607	614	620	627	633	640	646	653	659	666
	71	672	679	685	692	698	705	711	718	724	730
	72	737	743	750	756	763	769	776	782	789	795
	73	802	808	814	821	827	834	840	847	853	860
	74	866	872	879	885	892	898	905	911	918	924
	75	930	937	943	950	956	963	969	975	982	988
	76	82 995	*001	*008	*014	*020	*027	*033	*040	*046	*052
	77	83 059	065	072	078	085	091	097	104	110	117
	78	123	129	136	142	149	155	161	168	174	181
	79	187	193	200	206	213	219	225	232	238	245
	680	251	257	264	270	276	283	289	296	302	308
	81	315	321	327	334	340	347	353	359	366	372
	82	378	385	391	398	404	410	417	423	429	436
	83	442	448	455	461	467	474	480	487	493	499
	84	506	512	518	525	531	537	544	550	556	563
	85	569	575	582	588	594	601	607	613	620	626
	86	632	639	645	651	658	664	670	677	683	689
	87	696	702	708	715	721	727	734	740	746	753
	88	759	765	771	778	784	790	797	803	809	816
	89	822	828	835	841	847	853	860	866	872	879
	690	885	891	897	904	910	916	923	929	935	942
	91	83 948	954	960	967	973	979	985	992	998	*004
	92	84 011	017	023	029	036	042	048	055	061	067
	93	073	080	086	092	098	105	111	117	123	130
	94	136	142	148	155	161	167	173	180	186	192
	95	198	205	211	217	223	230	236	242	248	255
	96	261	267	273	280	286	292	298	305	311	317
	97	323	330	336	342	348	354	361	367	373	379
	98	386	392	398	404	410	417	423	429	435	442
	99	448	454	460	466	473	479	485	491	497	504
	700	84 510	516	522	528	535	541	547	553	559	566
Prop. Parts	N	0	1	2	3	4	5	6	7	8	9

	7
1	0.7
2	1.4
3	2.1
4	2.8
5	3.5
6	4.2
7	4.9
8	5.6
9	6.3

	6
1	0.6
2	1.2
3	1.8
4	2.4
5	3.0
6	3.6
7	4.2
8	4.8
9	5.4

N	0	1	2	3	4	5	6	7	8	9	Prop. Parts
700	84 510	516	522	528	535	541	547	553	559	566	
01	572	578	584	590	597	603	609	615	621	628	
02	634	640	646	652	658	665	671	677	683	689	
03	696	702	708	714	720	726	733	739	745	751	
04	757	763	770	776	782	788	794	800	807	813	
05	819	825	831	837	844	850	856	862	868	874	
06	880	887	893	899	905	911	917	924	930	936	
07	84 942	948	954	960	967	973	979	985	991	997	**7** **1** 0.7 **2** 1.4 **3** 2.1 **4** 2.8 **5** 3.5 **6** 4.2 **7** 4.9 **8** 5.6 **9** 6.3
08	85 003	009	016	022	028	034	040	046	052	058	
09	065	071	077	083	089	095	101	107	114	120	
710	126	132	138	144	150	156	163	169	175	181	
11	187	193	199	205	211	217	224	230	236	242	
12	248	254	260	266	272	278	285	291	297	303	
13	309	315	321	327	333	339	345	352	358	364	
14	370	376	382	388	394	400	406	412	418	425	
15	431	437	443	449	455	461	467	473	479	485	
16	491	497	503	509	516	522	528	534	540	546	
17	552	558	564	570	576	582	588	594	600	606	
18	612	618	625	631	637	643	649	655	661	667	
19	673	679	685	691	697	703	709	715	721	727	
720	733	739	745	751	757	763	769	775	781	788	
21	794	800	806	812	818	824	830	836	842	848	
22	854	860	866	872	878	884	890	896	902	908	
23	914	920	926	932	938	944	950	956	962	968	**6** **1** 0.6 **2** 1.2 **3** 1.8 **4** 2.4 **5** 3.0 **6** 3.6 **7** 4.2 **8** 4.8 **9** 5.4
24	85 974	980	986	992	998	*004	*010	*016	*022	*028	
25	86 034	040	046	052	058	064	070	076	082	088	
26	094	100	106	112	118	124	130	136	141	147	
27	153	159	165	171	177	183	189	195	201	207	
28	213	219	225	231	237	243	249	255	261	267	
29	273	279	285	291	297	303	308	314	320	326	
730	332	338	344	350	356	362	368	374	380	386	
31	392	398	404	410	415	421	427	433	439	445	
32	451	457	463	469	475	481	487	493	499	504	
33	510	516	522	528	534	540	546	552	558	564	
34	570	576	581	587	593	599	605	611	617	623	
35	629	635	641	646	652	658	664	670	676	682	
36	688	694	700	705	711	717	723	729	735	741	
37	747	753	759	764	770	776	782	788	794	800	
38	806	812	817	823	829	835	841	847	853	859	
39	864	870	876	882	888	894	900	906	911	917	**5** **1** 0.5 **2** 1.0 **3** 1.5 **4** 2.0 **5** 2.5 **6** 3.0 **7** 3.5 **8** 4.0 **9** 4.5
740	923	929	935	941	947	953	958	964	970	976	
41	86 982	988	994	999	*005	*011	*017	*023	*029	*035	
42	87 040	046	052	058	064	070	075	081	087	093	
43	099	105	111	116	122	128	134	140	146	151	
44	157	163	169	175	181	186	192	198	204	210	
45	216	221	227	233	239	245	251	256	262	268	
46	274	280	286	291	297	303	309	315	320	326	
47	332	338	344	349	355	361	367	373	379	384	
48	390	396	402	408	413	419	425	431	437	442	
49	448	454	460	466	471	477	483	489	495	500	
750	87 506	512	518	523	529	535	541	547	552	558	
N	0	1	2	3	4	5	6	7	8	9	Prop. Parts

N	0	1	2	3	4	5	6	7	8	9
750	87 506	512	518	523	529	535	541	547	552	558
51	564	570	576	581	587	593	599	604	610	616
52	622	628	633	639	645	651	656	662	668	674
53	679	685	691	697	703	708	714	720	726	731
54	737	743	749	754	760	766	772	777	783	789
55	795	800	806	812	818	823	829	835	841	846
56	852	858	864	869	875	881	887	892	898	904
57	910	915	921	927	933	938	944	950	955	961
58	87 967	973	978	984	990	996	*001	*007	*013	*018
59	88 024	030	036	041	047	053	058	064	070	076
760	081	087	093	098	104	110	116	121	127	133
61	138	144	150	156	161	167	173	178	184	190
62	195	201	207	213	218	224	230	235	241	247
63	252	258	264	270	275	281	287	292	298	304
64	309	315	321	326	332	338	343	349	355	360
65	366	372	377	383	389	395	400	406	412	417
66	423	429	434	440	446	451	457	463	468	474
67	480	485	491	497	502	508	513	519	525	530
68	536	542	547	553	559	564	570	576	581	587
69	593	598	604	610	615	621	627	632	638	643
770	649	655	660	666	672	677	683	689	694	700
71	705	711	717	722	728	734	739	745	750	756
72	762	767	773	779	784	790	795	801	807	812
73	818	824	829	835	840	846	852	857	863	868
74	874	880	885	891	897	902	908	913	919	925
75	930	936	941	947	953	958	964	969	975	981
76	88 986	992	997	*003	*009	*014	*020	*025	*031	*037
77	89 042	048	053	059	064	070	076	081	087	092
78	098	104	109	115	120	126	131	137	143	148
79	154	159	165	170	176	182	187	193	198	204
780	209	215	221	226	232	237	243	248	254	260
81	265	271	276	282	287	293	298	304	310	315
82	321	326	332	337	343	348	354	360	365	371
83	376	382	387	393	398	404	409	415	421	426
84	432	437	443	448	454	459	465	470	476	481
85	487	492	498	504	509	515	520	526	531	537
86	542	548	553	559	564	570	575	581	586	592
87	597	603	609	614	620	625	631	636	642	647
88	653	658	664	669	675	680	686	691	697	702
89	708	713	719	724	730	735	741	746	752	757
790	763	768	774	779	785	790	796	801	807	812
91	818	823	829	834	840	845	851	856	862	867
92	873	878	883	889	894	900	905	911	916	922
93	927	933	938	944	949	955	960	966	971	977
94	89 982	988	993	998	*004	*009	*015	*020	*026	*031
95	90 037	042	048	053	059	064	069	075	080	086
96	091	097	102	108	113	119	124	129	135	140
97	146	151	157	162	168	173	179	184	189	195
98	200	206	211	217	222	227	233	238	244	249
99	255	260	266	271	276	282	287	293	298	304
800	90 309	314	320	325	331	336	342	347	352	358
N	0	1	2	3	4	5	6	7	8	9

Prop. Parts

	6
1	0.6
2	1.2
3	1.8
4	2.4
5	3.0
6	3.6
7	4.2
8	4.8
9	5.4

	5
1	0.5
2	1.0
3	1.5
4	2.0
5	2.5
6	3.0
7	3.5
8	4.0
9	4.5

Prop. Parts

N	0	1	2	3	4	5	6	7	8	9
800	90 309	314	320	325	331	336	342	347	352	358
01	363	369	374	380	385	390	396	401	407	412
02	417	423	428	434	439	445	450	455	461	466
03	472	477	482	488	493	499	504	509	515	520
04	526	531	536	542	547	553	558	563	569	574
05	580	585	590	596	601	607	612	617	623	628
06	634	639	644	650	655	660	666	671	677	682
07	687	693	698	703	709	714	720	725	730	736
08	741	747	752	757	763	768	773	779	784	789
09	795	800	806	811	816	822	827	832	838	843
810	849	854	859	865	870	875	881	886	891	897
11	902	907	913	918	924	929	934	940	945	950
12	90 956	961	966	972	977	982	988	993	998	*004
13	91 009	014	020	025	030	036	041	046	052	057
14	062	068	073	078	084	089	094	100	105	110
15	116	121	126	132	137	142	148	153	158	164
16	169	174	180	185	190	196	201	206	212	217
17	222	228	233	238	243	249	254	259	265	270
18	275	281	286	291	297	302	307	312	318	323
19	328	334	339	344	350	355	360	365	371	376
820	381	387	392	397	403	408	413	418	424	429
21	434	440	445	450	455	461	466	471	477	482
22	487	492	498	503	508	514	519	524	529	535
23	540	545	551	556	561	566	572	577	582	587
24	593	598	603	609	614	619	624	630	635	640
25	645	651	656	661	666	672	677	682	687	693
26	698	703	709	714	719	724	730	735	740	745
27	751	756	761	766	772	777	782	787	793	798
28	803	808	814	819	824	829	834	840	845	850
29	855	861	866	871	876	882	887	892	897	903
830	908	913	918	924	929	934	939	944	950	955
31	91 960	965	971	976	981	986	991	997	*002	*007
32	92 012	018	023	028	033	038	044	049	054	059
33	065	070	075	080	085	091	096	101	106	111
34	117	122	127	132	137	143	148	153	158	163
35	169	174	179	184	189	195	200	205	210	215
36	221	226	231	236	241	247	252	257	262	267
37	273	278	283	288	293	298	304	309	314	319
38	324	330	335	340	345	350	355	361	366	371
39	376	381	387	392	397	402	407	412	418	423
840	428	433	438	443	449	454	459	464	469	474
41	480	485	490	495	500	505	511	516	521	526
42	531	536	542	547	552	557	562	567	572	578
43	583	588	593	598	603	609	614	619	624	629
44	634	639	645	650	655	660	665	670	675	681
45	686	691	696	701	706	711	716	722	727	732
46	737	742	747	752	758	763	768	773	778	783
47	788	793	799	804	809	814	819	824	829	834
48	840	845	850	855	860	865	870	875	881	886
49	891	896	901	906	911	916	921	927	932	937
850	92 942	947	952	957	962	967	973	978	983	988
N	0	1	2	3	4	5	6	7	8	9

Prop. Parts

	6
1	0.6
2	1.2
3	1.8
4	2.4
5	3.0
6	3.6
7	4.2
8	4.8
9	5.4

	5
1	0.5
2	1.0
3	1.5
4	2.0
5	2.5
6	3.0
7	3.5
8	4.0
9	4.5

Prop. Parts

Prop. Parts	N	0	1	2	3	4	5	6	7	8	9
	850	92 942	947	952	957	962	967	973	978	983	988
	51	92 993	998	*003	*008	*013	*018	*024	*029	*034	*039
	52	93 044	049	054	059	064	069	075	080	085	090
	53	095	100	105	110	115	120	125	131	136	141
	54	146	151	156	161	166	171	176	181	186	192
	55	197	202	207	212	217	222	227	232	237	242
6	56	247	252	258	263	268	273	278	283	288	293
1 0.6	57	298	303	308	313	318	323	328	334	339	344
2 1.2	58	349	354	359	364	369	374	379	384	389	394
3 1.8	59	399	404	409	414	420	425	430	435	440	445
4 2.4 **5** 3.0 **6** 3.6	**860**	450	455	460	465	470	475	480	485	490	495
7 4.2	61	500	505	510	515	520	526	531	536	541	546
8 4.8	62	551	556	561	566	571	576	581	586	591	596
9 5.4	63	601	606	611	616	621	626	631	636	641	646
	64	651	656	661	666	671	676	682	687	692	697
	65	702	707	712	717	722	727	732	737	742	747
	66	752	757	762	767	772	777	782	787	792	797
	67	802	807	812	817	822	827	832	837	842	847
	68	852	857	862	867	872	877	882	887	892	897
	69	902	907	912	917	922	927	932	937	942	947
	870	93 952	957	962	967	972	977	982	987	992	997
	71	94 002	007	012	017	022	027	032	037	042	047
5	72	052	057	062	067	072	077	082	086	091	096
1 0.5 **2** 1.0	73	101	106	111	116	121	126	131	136	141	146
3 1.5	74	151	156	161	166	171	176	181	186	191	196
4 2.0 **5** 2.5	75	201	206	211	216	221	226	231	236	240	245
6 3.0	76	250	255	260	265	270	275	280	285	290	295
7 3.5	77	300	305	310	315	320	325	330	335	340	345
8 4.0	78	349	354	359	364	369	374	379	384	389	394
9 4.5	79	399	404	409	414	419	424	429	433	438	443
	880	448	453	458	463	468	473	478	483	488	493
	81	498	503	507	512	517	522	527	532	537	542
	82	547	552	557	562	567	571	576	581	586	591
	83	596	601	606	611	616	621	626	630	635	640
	84	645	650	655	660	665	670	675	680	685	689
	85	694	699	704	709	714	719	724	729	734	738
	86	743	748	753	758	763	768	773	778	783	787
	87	792	797	802	807	812	817	822	827	832	836
4	88	841	846	851	856	861	866	871	876	880	885
1 0.4 **2** 0.8	89	890	895	900	905	910	915	919	924	929	934
3 1.2 **4** 1.6	**890**	939	944	949	954	959	963	968	973	978	983
5 2.0	91	94 988	993	998	*002	*007	*012	*017	*022	*027	*032
6 2.4	92	95 036	041	046	051	056	061	066	071	075	080
7 2.8 **8** 3.2	93	085	090	095	100	105	109	114	119	124	129
9 3.6	94	134	139	143	148	153	158	163	168	173	177
	95	182	187	192	197	202	207	211	216	221	226
	96	231	236	240	245	250	255	260	265	270	274
	97	279	284	289	294	299	303	308	313	318	323
	98	328	332	337	342	347	352	357	361	366	371
	99	376	381	386	390	395	400	405	410	415	419
	900	95 424	429	434	439	444	448	453	458	463	468
Prop. Parts	**N**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

N	0	1	2	3	4	5	6	7	8	9	Prop. Parts
900	95 424	429	434	439	444	448	453	458	463	468	
01	472	477	482	487	492	497	501	506	511	516	
02	521	525	530	535	540	545	550	554	559	564	
03	569	574	578	583	588	593	598	602	607	612	
04	617	622	626	631	636	641	646	650	655	660	
05	665	670	674	679	684	689	694	698	703	708	
06	713	718	722	727	732	737	742	746	751	756	
07	761	766	770	775	780	785	789	794	799	804	
08	809	813	818	823	828	832	837	842	847	852	
09	856	861	866	871	875	880	885	890	895	899	
910	904	909	914	918	923	928	933	938	942	947	
11	952	957	961	966	971	976	980	985	990	995	
12	95 999	*004	*009	*014	*019	*023	*028	*033	*038	*042	**5** **1** 0.5 **2** 1.0 **3** 1.5 **4** 2.0 **5** 2.5 **6** 3.0 **7** 3.5 **8** 4.0 **9** 4.5
13	96 047	052	057	061	066	071	076	080	085	090	
14	095	099	104	109	114	118	123	128	133	137	
15	142	147	152	156	161	166	171	175	180	185	
16	190	194	199	204	209	213	218	223	227	232	
17	237	242	246	251	256	261	265	270	275	280	
18	284	289	294	298	303	308	313	317	322	327	
19	332	336	341	346	350	355	360	365	369	374	
920	379	384	388	393	398	402	407	412	417	421	
21	426	431	435	440	445	450	454	459	464	468	
22	473	478	483	487	492	497	501	506	511	515	
23	520	525	530	534	539	544	548	553	558	562	
24	567	572	577	581	586	591	595	600	605	609	
25	614	619	624	628	633	638	642	647	652	656	
26	661	666	670	675	680	685	689	694	699	703	
27	708	713	717	722	727	731	736	741	745	750	
28	755	759	764	769	774	778	783	788	792	797	
29	802	806	811	816	820	825	830	834	839	844	
930	848	853	858	862	867	872	876	881	886	890	
31	895	900	904	909	914	918	923	928	932	937	
32	942	946	951	956	960	965	970	974	979	984	4 **1** 0.4 **2** 0.8 **3** 1.2 **4** 1.6 **5** 2.0 **6** 2.4 **7** 2.8 **8** 3.2 **9** 3.6
33	96 988	993	997	*002	*007	*011	*016	*021	*025	*030	
34	97 035	039	044	049	053	058	063	067	072	077	
35	081	086	090	095	100	104	109	114	118	123	
36	128	132	137	142	146	151	155	160	165	169	
37	174	179	183	188	192	197	202	206	211	216	
38	220	225	230	234	239	243	248	253	257	262	
39	267	271	276	280	285	290	294	299	304	308	
940	313	317	322	327	331	336	340	345	350	354	
41	359	364	368	373	377	382	387	391	396	400	
42	405	410	414	419	424	428	433	437	442	447	
43	451	456	460	465	470	474	479	483	488	493	
44	497	502	506	511	516	520	525	529	534	539	
45	543	548	552	557	562	566	571	575	580	585	
46	589	594	598	603	607	612	617	621	626	630	
47	635	640	644	649	653	658	663	667	672	676	
48	681	685	690	695	699	704	708	713	717	722	
49	727	731	736	740	745	749	754	759	763	768	
950	97 772	777	782	786	791	795	800	804	809	813	
N	0	1	2	3	4	5	6	7	8	9	Prop. Parts

Prop. Parts	N	0	1	2	3	4	5	6	7	8	9
	950	97 772	777	782	786	791	795	800	804	809	813
	51	818	823	827	832	836	841	845	850	855	859
	52	864	868	873	877	882	886	891	896	900	905
	53	909	914	918	923	928	932	937	941	946	950
	54	97 955	959	964	968	973	978	982	987	991	996
	55	98 000	005	009	014	019	023	028	032	037	041
	56	046	050	055	059	064	068	073	078	082	087
	57	091	096	100	105	109	114	118	123	127	132
	58	137	141	146	150	155	159	164	168	173	177
	59	182	186	191	195	200	204	209	214	218	223
	960	227	232	236	241	245	250	254	259	263	268
	61	272	277	281	286	290	295	299	304	308	313
5 **1** 0.5 **2** 1.0 **3** 1.5 **4** 2.0 **5** 2.5 **6** 3.0 **7** 3.5 **8** 4.0 **9** 4.5	62	318	322	327	331	336	340	345	349	354	358
	63	363	367	372	376	381	385	390	394	399	403
	64	408	412	417	421	426	430	435	439	444	448
	65	453	457	462	466	471	475	480	484	489	493
	66	498	502	507	511	516	520	525	529	534	538
	67	543	547	552	556	561	565	570	574	579	583
	68	588	592	597	601	605	610	614	619	623	628
	69	632	637	641	646	650	655	659	664	668	673
	970	677	682	686	691	695	700	704	709	713	717
	71	722	726	731	735	740	744	749	753	758	762
	72	767	771	776	780	784	789	793	798	802	807
	73	811	816	820	825	829	834	838	843	847	851
	74	856	860	865	869	874	878	883	887	892	896
	75	900	905	909	914	918	923	927	932	936	941
	76	945	949	954	958	963	967	972	976	981	985
	77	98 989	994	998	*003	*007	*012	*016	*021	*025	*029
	78	99 034	038	043	047	052	056	061	065	069	074
	79	078	083	087	092	096	100	105	109	114	118
	980	123	127	131	136	140	145	149	154	158	162
	81	167	171	176	180	185	189	193	198	202	207
4 **1** 0.4 **2** 0.8 **3** 1.2 **4** 1.6 **5** 2.0 **6** 2.4 **7** 2.8 **8** 3.2 **9** 3.6	82	211	216	220	224	229	233	238	242	247	251
	83	255	260	264	269	273	277	282	286	291	295
	84	300	304	308	313	317	322	326	330	335	339
	85	344	348	352	357	361	366	370	374	379	383
	86	388	392	396	401	405	410	414	419	423	427
	87	432	436	441	445	449	454	458	463	467	471
	88	476	480	484	489	493	498	502	506	511	515
	89	520	524	528	533	537	542	546	550	555	559
	990	564	568	572	577	581	585	590	594	599	603
	91	607	612	616	621	625	629	634	638	642	647
	92	651	656	660	664	669	673	677	682	686	691
	93	695	699	704	708	712	717	721	726	730	734
	94	739	743	747	752	756	760	765	769	774	778
	95	782	787	791	795	800	804	808	813	817	822
	96	826	830	835	839	843	848	852	856	861	865
	97	870	874	878	883	887	891	896	900	904	909
	98	913	917	922	926	930	935	939	944	948	952
	99	99 957	961	965	970	974	978	983	987	991	996
	1000	00 000	004	009	013	017	022	026	030	035	039
Prop. Parts	**N**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**

AUTHOR INDEX

Abderhalden, E., 4, 59, 149, 208
Abrahamczik, E., 49, 137, 146, 179, 281, 289
Adams, J. E., 146
Adams, R., 274, 293
Adler, S., 98, 99
Afanasev, B. N., 208
Ainsworth, A. W., 12
Aisenstadt, J., 197, 204
Alber, H. K., 3, 12, 35, 40, 49, 65, 208, 289, 291, 293
Alicino, J. F., 96, 99, 100, 197
Allen, C. F. H., 99
Allen, R. M., 291
Altern, F., 179
Ambler, H. R., 148
Amdur, E., 293
Andrews, L. W., 179
Angeletti, A., 197
Ångström, A. T., 13
Arndt, F., 248, 250, 271
Asahina, Y., 138, 146
Autenrieth, W., 179
Auwers, K., 251
Avery, S., 146

Backeberg, O. G., 146
Baernstein, H. D., 251
Bailey, A. J., 197, 261, 262
Baker, R. H., 291
Ball, T. Z., 146
Bang, I., 59, 76, 77, 179
Bannai, S., 138, 146
Barger, G., 1, 3, 237, 238
Barkenbus, C., 291
Barrenscheen, H. K., 204
Bartosiewicz, S. Z., 77
Batt, W. G., 291
Baubigny, H., 176, 179
Baule, B., 49
Baum, H., 4, 180, 198
Baumeister, W., 275
Baxley, Wm. H., 177, 180
Baxter, G. P., 146
Beamish, F. E., 177, 179, 197, 208
Beaucourt, K., 96, 99, 133, 146
Beazley, C. W., 177, 179, 192, 197
Beet, A. E., 77
Behrens, H., 281, 291
Belcher, R., 77
Bell, D. J., 274
Benedetti-Pichler, A. A., 4, 13, 31, 32, 40, 49, 60, 100, 148, 180, 205, 207, 208, 228, 250, 251, 281, 282, 291, 292
Berend, M., 176, 179
Berger, H., 146, 179
Berl, E., 96, 99, 146, 238
Bernal, J. D., 292
Berraz, G., 99, 146
Berry, A. J., 179
Beutler, R., 292
Biltz, H., 228
Blackman, P., 228
Blade, E., 31
Blank, E. W., 228, 292
Blanke, E., 276
Bleier, F., 228
Block, R. J., 68
Blount, B. K., 292
Booth, H. S., 228
Blümel, F., 49, 179, 281, 289
Blumer, F., 137, 146
Bobranski, B., 146, 149, 212, 213, 214, 215, 216
Böck, F., 96, 99, 133, 146
Böe, J., 180
Boell, E. J., 77
Boetius, M., 4, 133, 134, 146, 281
Böhme, H., 220
Boivin, A., 139, 146, 148
Booth, H. S., 31
Bordeianu, C. V., 179
Börnstein, R., 228
Borsche, W., 179

Brackenburg, J., 146
Brant, J. H., 77
Bratton, A. C., 179, 228
Brecher, C., 181, 209, 262
Breitner, P., 35
Brenner, M., 250, 251
Bretschneider, H., 275
Breuer, F., 13, 31, 35, 50, 97, 99
Brinton, L. H., 31
Britzke, E. V., 50
Brodie, S. S., 99, 146, 148
Brody, E., 99
Broun, D., 179
Brown, J. W., 289
Browning, B. L., 292
Bruce, W. F., 146
Bruckner, V., 248, 251
Brunck, O., 197
Bryant, J. T., 291
Buchholz, J., 179
Buhn, T., 251
Bullock, B., 179
Burdett, R. A., 179
Burg, W. V., 197
Burger, G., 275
Bürger, K., 136, 146, 149, 177, 179, 207, 209
Burkhart, H., 96, 99, 146
Busbey, R. L., 179
Busch, M., 177, 179
Butler, A. Q., 179
Buttescu, D., 177, 179

Campbell, N., 292
Canal, F., 179
Canessa, J. C., 65
Carius, L., 179, 190, 197
Cass, Wm. E., 35
Cassidy, H. G., 262
Caulfield, T. H., 197, 251
Chablay, E., 179
Chamot, É. M., 281, 292
Chaney, A. S., 60
Chapin, T., 228
Chavanne, G., 176, 179
Cherbuliez, E., 99
Chinoy, J. J., 247, 251
Cholnoky, L., 295
Chou, T. P., 220
Christensen, B. E., 146, 208, 247, 251
Christian, W., 276
Christiansen, J. A., 208
Ciochina, J., 292
Clark, E. P., 99, 146, 179, 197, 247, 249, 251, 261, 262
Clark, H. S., 148
Clark, R. O., 137, 146
Clarke, B. L., 13, 281, 289
Clarke, H. T., 292
Clemo, G. R., 292
Clutterbuck, P. W., 271
Cohen, J., 78
Colegrave, E. B., 292
Collander, R., 292
Collins, H. L., 208
Collot, A., 13
Colson, A. F., 247, 251
Conner, E. B., 148
Conway, E. J., 60
Coombs, H. I., 4, 65
Cornwell, R. T. K., 96, 99, 137, 146
Corwin, A. H., 4, 12, 13, 31, 35, 146
Craig, A., 13
Craig, L. C., 292
Craig, R., 195, 198
Crowfoot, D., 292

Dadieu, A., 292
Damerell, R. V., 31
Danford, V., 148
Das Gupta, H. N., 77, 179
Daubney, C. G., 179
Davenport, J. E., 147, 271
Daw, E. B., 4, 32, 41, 50, 61, 65, 68, 77, 100, 149, 181, 198, 204, 209, 216, 220, 229, 238, 251, 262, 271, 275, 282, 294
DeCeuster, P., 228
Degering, E. F., 146
Delbridge, T. G., 179
Dellavilla, M., 179
Dennis, L. M., 292
Dennstedt, M., 138, 146, 177, 179, 191, 197
Derner, O. C., 292
Derner, V. H., 292
Deturk, E. E., 78
Devillers, G., 208
Diepolder, E., 96, 99
Dieterle, H., 139, 146, 176, 179
Dijk, C. P., 181

Dinelli, D., 192, 198
Dittmer, K. H., 275
Doan, D. J., 137, 149
Dobromyslova, A. V., 272
Dobrovolny, F. J., 148
Dod, K., 180
Doering, H., 179
Doeuvre, J., 275
Donau, J., 1, 4, 12, 13, 179, 190, 197, 275, 292
Dostal, V., 179
Drake, N. L., 179
Drew-Keith, H. D., 146, 179, 208
Dubsky, J. V., 96, 99, 137, 146, 289
Dumas, A., 228
Dumazert, C., 77
Dunbar, R. E., 179, 197, 292
Dziobeck, L., 65

Eaton, F. C., 31
Eckstrand, G., 229
Edwards, A. E., 292
Ehret, W. F., 14, 150
Eigenberger, E., 99
Eisner, A., 77
Eisner, W., 60
Eitingon, M., 4, 61, 68, 77
Elek, A., 3, 4, 50, 60, 75, 77, 132, 146, 179, 193, 197, 203, 204, 247, 248, 251, 261, 262
Elek, S. D., 41
Elsner, H., 275
Embden, G., 204
Emich, F., 1, 3, 4, 12, 13, 41, 50, 60, 176, 179, 190, 193, 197, 228, 281, 282, 292
Endies, G., 179
Engelberg, H., 181
Engelder, C. J., 281
Erdös, J., 292
Errara, L., 237
Escourrou, R., 275
Evans, R. N., 147, 271
Ewing, D. T., 275

Facer, J. F., 208
Fajans, K., 179
Fales, H. A., 31
Fang, H. Y., 220
Feigl, F., 204
Feldman, H. B., 180
Fellenberg, Th. von, 175, 177, 180
Ferguson, G. E., 50, 77
Fieser, L. F., 147
Fife, J. M., 77, 292
Fine, J., 292
Fischer, E., 275, 292
Fischer, F. G., 275
Fischer, H., 97, 99
Fisher, H. L., 147, 220
Fisher, R. S., 198
Flaschenträger, B., 31, 98, 99, 137, 147, 270, 271, 292
Flatt, R., 60
Folch, J., 3, 5, 140
Foresti, B., 275
Forsee, W. T., 148
Foulke, D. G., 292, 294
Francis, L. D., 292
Frazer, J. C., 208
Freed, M., 262
French, R. B., 275
Freudenberg, K., 60, 261, 262, 275
Friedman, A. F., 76, 77
Friedman, T. E., 147
Friedrich, A., 3, 4, 35, 41, 50, 60, 65, 75, 77, 96, 99, 132, 134, 135, 136, 137, 147, 180, 191, 196, 197, 204, 216, 220, 237, 238, 248, 249, 251, 261, 262, 282
Fuchs, H. J., 60, 61, 78
Fuchs, L., 13
Fuchs, W., 271
Fuller, M. L., 61
Fulton, R. W., 292
Funk, C., 99, 147
Fünner, W., 147
Furman, N. H., 32, 60
Furter, M., 13, 68, 147, 251, 292
Fuson, R. C., 271, 294
Fyleman, E., 4, 100, 204, 229

Gagarin, R., 292
Garner, W., 282, 292
Geilman, W., 60
Gelman, N. E., 197
Gessner, E., 181
Gettens, R. J., 292
Gettler, A. O., 250, 251, 292
Gibbs, G. E., 77
Gibson, D. J., 197, 251

Gibson, G. L., 293
Gicklhorn, J., 289
Gill, A. H., 293
Glockler, G., 208
Gloss, K., 180
Goldberg, L. J., 180
Goodloe, P., 208
Goodwin, L. F., 275
Gorbach, G., 12, 13, 293
Govaert, F., 99
Gränacher, C., 99, 147
Grant, J., 197
Grant, K., 14
Grassmann, W., 68
Grassner, F., 50, 100, 282, 289
Green, J. R., 77
Grey, E. C., 282
Griebel, C., 293
Griffel, M., 180, 198
Grignard, V., 275
Grote, W., 180, 197
Grün, A., 275
Grünsteidl, E., 50
Grüntzig, W., 294
Gubser, H., 68
Gustavson, R. G., 275
Gutzeit, G., 282
Guzman, J., 60

Haagen-Smit, A. J., 275
Haas, P., 136, 147
Haberland, G., 251
Hagedorn, H. C., 60
Hahn, F. L., 60
Hale, A. H., 146
Halla, F., 99
Hallett, L. T., 77, 137, 147, 177, 180, 192, 197
Hamill, W. H., 99, 134, 147
Hammond, W. A., 147
Harand, J., 13, 208, 289
Harries, C., 275
Harries, L. J., 68
Harries, T. H., 98, 100
Harris, G. W., 31
Harrison, K., 274
Harte, R. A., 3, 4, 75, 77, 179, 261, 262
Hartley, O., 77
Hartmann, A. F., 61
Hayman, D. F., 31, 50, 98, 99, 132, 147, 293
Hays, E. E., 293
Heatley, N. G., 60
Hecht, F., 41, 147
Hefter, O., 238
Heilbron, I. M., 293
Heimburger, G., 179
Hein, F., 96, 99
Heller, K., 31, 65, 197
Hellmann, J., 60
Henderson, W. E., 228
Hendricks, S. B., 293
Henne, A. L., 180, 181
Hennig, H., 147, 150, 198
Hepner, B., 147
Hermance, H. W., 13, 289
Hernler, F., 31, 50, 99, 147, 175, 180
Hershberg, E. B., 97, 99, 100
Herzig, T., 251
Heslinga, J., 149, 177, 181, 193, 198, 207, 209, 282
Hetterich, H., 293
Heuser, G. F., 276
Heyde, W., 68
Hilbck, H., 293
Hilbert, H., 271
Hill, D. W., 3, 4, 179, 193, 197, 203, 204
Hille, E., 179
Hilty, W. W., 180
Hoeltje, R., 60
Hoffmann, E., 50
Hofmann, A. W., 228
Hohenberg, E., 181
Hohl, H. V., 147
Hohn, H., 275
Holter, H., 60
Hoover, A., 60
Hopkins, A., 31
Houben, J., 180, 197, 271
Hubbard, D. M., 180
Huffman, E. W. D., 146, 147, 192, 197
Huntress, E. H., 293
Hurley, F. H., 31
Hyde, J. F., 275

Ide, W. S., 96, 100
Ihmori, T., 14
Ilberg, K., 294
Ishler, N. H., 271

Itano, I., 180
Ivanei, I. F., 208
Iverson, P., 204

Jackson, H., 275
Jacobsen, R. P., 147
Jacobson, P., 294
Jacquemain, R., 208
Jefferson, M. E., 293
Jelley, E. E., 293
Jendrassik, L., 65, 293
Jensen, B. N., 60
Johns, I. B., 13, 137, 147, 198
Johnson, A. H., 77
Johnson, D. L., 60
Jones, H. C., 228
Jones, R. N., 275
Josephson, B., 198
Jouriaux, M., 220
Judefind, W. L., 275

Kagan, S. L., 60
Kahane, E., 198
Kahane, M., 198
Kahler, H. L., 198
Kahovec, L., 248, 251
Kamio, S., 178, 180
Kamm, O., 293
Karrer, P., 275
Katow, I. A., 60
Kaufman, L., 179
Kautsky, H., 275
Kelber, C., 180
Kemmerer, G., 77, 137, 147
Kemper, W. A., 198
Kempf, R., 293
Kendall, A. I., 147
Kendall, E. C., 177, 180
Kerschbaum, E., 208, 262
Khouri, J., 60
Kimball, R. H., 177, 180
Kinsman, S., 251
Kirby, H., 147
Kirk, M. M., 275
Kirk, P. L., 41, 60, 75, 77, 136, 147, 179, 180, 195, 198, 203, 204, 289, 293
Kirkish, F. J., 60
Kirner, W. R., 13, 32, 50, 68, 135, 139, 147, 149, 208, 289
Kirpal, A., 251
Kisser, J., 293
Kitamura, R., 198
Kjeldahl, J., 77
Klein, G., 293
Kley, F., 281, 291
Knoll, A., 41
Koegel, R., 65, 180, 198
Koerber, W., 146
Kofler, L., 293
Kögl, F., 262
Kohler, E. P., 270, 271
Kohn, L., 228
Kolthoff, I. M., 32, 60, 180, 275, 293
Kondo, Y., 293
Kopfer, F., 147
Köppen, R., 275
Kopper, H., 292
Korenman, I. M., 60, 180
Kostrov, I. V., 180
Kraemer, G., 100
Krainick, H. G., 3, 5, 50, 61, 148, 176, 181, 208
Kraus, H., 198
Krause, W., 60
Krebs, K. G., 262
Kreider, L. C., 13
Krekeler, H., 180, 197
Krellwitz, L., 271
Kröcker, F., 96, 100, 148, 208
Krogh, A., 147
Krumholz, D., 60
Kuck, J. A., 4, 12, 13, 147, 180, 198
Kuespert, K. H., 148
Kuhlmann, Wm. H. F., 1, 7, 12, 14
Kuhn, R., 3, 4, 204, 251, 261, 262, 275
Kuipers, J. W., 192, 197
Künster, W., 251
Kurzen, G., 149
Küster, F. W., 220
Kutter, F., 293

Labriola, R. A., 148, 150
LaCoste, W., 228
Lacourt, A., 180, 208
Landis, Q., 180
Landolt, H., 228
Lange, L., 148
Lange, N. A., 293
Larson, K. O., 180
Laskin, I. E., 60

Lauer, W. M., 100, 148
Lauro, M. F., 77
Lax, H., 275
Leclerq, L., 180
Lehrecke, H., 293
Leipert, T., 175, 180, 208, 251, 262
Lenz, W., 13
Levy, A. M., 4, 238
Lieb, H., 50, 136, 139, 148, 202, 204, 208, 251, 282, 289
Lieff, M., 247, 251
Limprecht, H., 100
Lin, I., 32
Lindenfeld, K., 137, 148, 198, 289
Linderström-Lang, K., 60
Lindner, J., 4, 50, 60, 133, 134, 139, 148, 208
Lindner, R., 203, 204
Lingane, J. J., 275
Links, R., 60
Lisle, E. B., 251
Liu, Y. P., 220
Livingstone, E. M., 136, 148
Ljungdahl, M., 60
Llacer, A., 207, 208
Lochte, H. L., 60, 228
Löwenburg, K., 275
Loewenstein, E., 12, 13
Lomholt, S., 208
Lorenz, v., N., 204
Lucas, R., 50, 100
Lumsden, T. S., 228
Lunde, G., 148, 175, 180
Lunge, G., 57, 60, 228
Lüttgens, W., 271

Maag, W., 251
Mabee, F. C., 282
Maclay, W., 146
MacNevin, W. M., 148, 177, 180
Mandl, F., 197
Manov, G. G., 198
Marks, M. E., 209
Marrian, G. F., 271
Marrian, P. M., 271
Mason, C. W., 281, 292
Mathewson, W. E., 181
Matthews, N. L., 180
Matukawa, D., 275
Mayr, C., 208, 262
Mayrhofer, A., 293
McCalla, A., 136, 147
McClendon, J. F., 179
McCoy, J. S., 4, 180, 198
McDonald, E., 289
McGreal, M. E., 293
McLean, A., 293
McQuillen, A., 292
Meadow, J. R., 138, 148
Medinskii, K. B., 180
Meeker, E. W., 77
Meisenheimer, J., 271
Meixner, A., 96, 100, 148, 208
Meloche, V. W., 294
Menzel, H., 60
Menzies, A. W. C., 2, 15, 216
Merz, K. W., 262
Messinger, J., 275
Meyer, H., 180, 198, 251, 262
Meyer, K., 31, 197
Meyer, V., 228
Middleton, H., 293
Mika, J., 50, 60
Miksch, R., 178, 180
Miller, C. C., 198
Miller, H. S., 77
Millner, T., 99
Milner, H. W., 216
Milner, R. T., 50, 98, 100, 138, 149, 215
Möller, E. F., 275
Morgulis, S., 76
Morton, A. A., 293
Moser, L., 178, 180
Moses, C. G., 275
Mouquin, H., 293
Moyer, H. V., 13
Müller, E., 50, 100, 134, 148
Müller, O. H., 275
Müller, R. H., 13, 148, 273, 275, 276
Mulliken, S. P., 293
Münster, W., 180
Murai, J., 181

Nachtwey, P., 271
Nagel, R. H., 4, 13, 32, 100, 139, 148 180
Nanji, H. R., 251
Nardo de, L. V., 148
Natelson, S., 99, 148, 293
Nath, M. C., 138, 148

Needham, J., 77
Negelein, E., 271
Nemes, T., 149, 209
Nernst, W., 1, 4, 12, 13, 228
Neuberg, O., 228
Neumann, F., 247, 248, 251
Neuworth, M., 271
Nichols, L., 293
Nichols, M. L., 98, 100
Nicloux, M., 77, 139, 148
Niederl, J. B., 4, 5, 13, 32, 41, 50, 57, 61, 65, 68, 77, 95, 97, 98, 99, 100, 136, 138, 139, 145, 148, 149, 175, 180, 198, 228, 238, 250, 251, 275, 276, 282, 289, 292, 293, 294
Niederl, V., 4, 13, 32, 61, 68, 77, 100, 148, 180
Niemann, C., 148
Noller, C. R., 251
Nomura, S., 181
Norton, A. R., 65, 149
Noyons, E., 294

Oakdale, V. O., 181
Ochiai, E., 181
Oddo, B., 271
Oerskov, S. L., 61
Ogawa, S., 96, 100
Oppler, B., 181
Orthner, L., 148
Osborn, R. A., 77
Otto, C. E., 292

Page, I. H., 275
Parnas, J. K., 77
Parr, S. W., 177, 181, 193, 198
Paulson, R. A., 13, 31
Pavelka, F., 41
Peabody, W. A., 198
Peak, D. A., 229
Pentschev, N. P., 100, 148
Perrot, A., 100
Petak, K., 181
Peterson, J. B., 289
Petras, P., 293
Pettenkoffer, P., 148
Petterson, O., 229
Pfenningberger, R., 175, 180
Phansalkar, S. L., 61
Pilch, F., 77
Pirie, N. W., 262
Pirsch, J., 50, 220
Piutti, P., 192, 198
Plentl, A., 4, 50, 228
Pojas, M., 147
Pollak, L., 292
Pollard, C. B., 148
Porter, C. R., 146, 208
Porter, C. W., 229
Postowsky, J. J., 262
Poth, E. J., 96, 100
Powell, A. L., 180
Power, F. W., 13, 133, 149
Prager, B., 294
Prater, A. N., 149, 275
Pregl, F., 1, 2, 4, 7, 13, 32, 34, 35, 36, 61, 65, 77, 82, 96, 98, 99, 100, 113, 132, 133, 134, 135, 136, 137, 138, 145, 149, 176, 181, 191, 196, 198, 204, 207, 209, 215, 216, 220, 229, 238, 247, 248, 250, 251, 261, 271, 275, 277, 281, 282, 294
Pringsheim, H., 198

Radlberger, L., 50
Ramberg, L., 12, 13
Rangaswami, S., 100
Rappaport, F., 136, 147, 181
Rast, K., 220, 237, 238
Rauscher, W. H., 176, 181
Redeman, C. E., 77
Rehberg, P. B., 61
Reichel, L., 148
Reid, E. E., 275
Reihlein, H., 149
Reith, J. F., 181
Renzenberg, M., 291
Retgers, J. W., 294
Reuter, F., 41
Revukas, A. J., 147, 271
Rezek, A., 216
Richards, T. W., 28, 32
Richter, R., 229
Richter-Quittner, M., 275
Rieche, A., 215, 216
Riesenfeld, E. H., 1, 4, 13, 149
Rigakos, D. R., 247, 251
Rischbieth, P., 149
Roberts, L. D., 208
Robertson, P. W., 181
Robinson, R. A., 229, 237, 238

Robinson, R. J., 137, 149, 261, 262
Rodden, C. J., 50, 65, 149
Rogers, H., 31
Ronzio, A. R., 100
Röscheisen, P., 35
Rosenblum, C., 294
Rosenmund, K. W., 181
Rosenthaler, L., 294
Roswell, C. A., 294
Roth, H., 3, 4, 32, 41, 50, 61, 65, 68, 77, 100, 132, 149, 181, 198, 204, 209, 216, 220, 229, 238, 251, 261, 262, 271, 275, 282, 294
Roth, R. T., 4, 148
Routh, I. B., 4, 228, 294
Royer, G. L., 65, 136, 149, 181, 198
Rudolph, W., 198
Rudy, H., 275
Ruebke, K., 181
Ruess, H., 293
Russel, W. W., 209

Sadavisan, V., 78
Sakamoto, H., 149, 178, 180, 198, 276
Sanchez, J. A., 149, 181
Sandell, E. B., 32
Sandhoff, A. G., 271
Sandza, J. G., 96, 100
Sargent, E. H., 149
Saschek, W., 3, 4, 5, 35, 100, 193, 198, 227, 228, 229
Saylor, C. P., 294
Schadendorf, E., 149
Schally, E., 294
Schaposchnikow, W. G., 77
Scheflan, L., 50, 77
Schenk, R., 149
Scheringa, K., 181
Schiller, W., 281
Schilow, E., 59, 61
Schleiermacher, A., 229, 294
Schlichenmaier, W., 271
Schlömer, A., 178, 181
Schmidt, A., 146
Schmitt, R. B., 4, 139, 149, 212, 213, 215, 216, 227, 229, 238, 272
Schneider, E., 220
Schneider, F., 4, 41, 50, 149, 197, 227, 228, 229, 282, 292, 294
Schöberl, A., 137, 181, 198
Schoeller, A., 100
Schöffel, E. W., 289
Schoorl, N., 149, 294
Schroeder, W. C., 198
Schuetze, M., 209
Schuhecker, K., 204
Schukal, J., 41
Schulek, E., 77
Schulze, P., 13
Schumacher, A. E., 276
Schupinskaja, M., 150
Schwarz, K., 61, 238
Schwarz-Bergkampf, E., 12, 13, 32, 178
Scott, E. W., 181
Scott, J. E., 78
Screenivasan, A., 78
Secareau, S., 41
Seeker, A. F., 181
Sepalowa-Michailowa, L. A., 149
Shaffer, P. A., 61
Shah, P. T., 220
Shea, F., 97, 100
Shead, A. C., 294
Sheen, R. T., 198
Shelberg, E. F., 289
Shelton, R. S., 292
Sherman, M. S., 50, 98, 100, 138, 149
Sherp, H. W., 275
Shiwow, W. C., 50
Shrewsbury, C. L., 60
Shriner, R. H., 294
Shriner, R. L., 274
Shtuber, E. Y., 272
Sievers, D. C., 77
Signer, R., 238
Silbert, E. P., 65
Silbert, F. C., 149
Simon, L. J., 276
Simms, H. S., 293, 294
Sinclair, D. A., 237, 238
Siwoloboff, A., 229, 291, 294
Sjoberg, K., 276
Sloof, A., 181
Slotta, K. H., 251, 276
Smith, J. H. C., 215, 216, 276
Sobel, A., 78
Sobotka, H., 75, 77
Soltys, A., 3, 5, 41, 50, 136, 148, 220, 261, 262, 270, 272, 289, 294
Sörensen, S. P. L., 61

Southworth, L., 100
Sozzi, J. A., 207, 208
Spatz, W., 61
Sperry, W. M., 35, 294
Spiess, J. R., 98, 100, 237, 238
Spikes, W. F., 40, 228, 281, 292
Spoerri, T. E., 282
Standen, G. W., 61
Stanke, V., 149, 209
Starkweather, H., 146
Stary, Z., 65
Staudinger, F., 294
Steele, B. D., 15
Stepanow, A., 176, 181
Stern, D., 294
Sternberg, H., 12, 15, 32, 35, 50, 147, 149
Stillson, G. H., 137, 146
Stodola, F. H., 262
Stoll, A., 251
Stone, J. F., 271
Stonestreet, G. O., 181
Strebinger, R., 50, 204, 209
Stross, M. J., 181
Struszynski, M., 294
Stubblefield, F. M., 78
Sucharda, E., 146, 149, 212, 213, 214, 215, 216
Sudborough, J. J., 271
Sundberg, O. E., 149
Sunde, C. J., 100
Svanberg, O., 276

Tamura, K., 294
Ter Meulen, H., 149, 177, 181, 193, 198, 207, 209, 282
Theilacker, W., 181
Thiel, A., 220
Thimann, K. V., 61
Thomis, C. N., 61
Thompson, J. J., 181
Thornton, W., 32
Thunberg, T., 61
Tiedcke, C., 50, 100, 138, 149, 181, 198, 220, 289
Titus, L., 294
Tomiček, O., 181
Trautz, O., 4, 5, 50, 96, 98, 100, 229
Tressler, D. K., 275
Tschugaeff, L., 270, 272
Tsuda, K., 276
Turner, R. R., 32

Umezawa, S., 208, 209
Unterzaucher, J., 50, 149, 181, 198, 207, 209
Utz, F., 181
Utzinger, M., 251

Vance, J. E., 134, 149
Van Mater, H. L., 149
Van Nieuwenburg, C. J., 205, 208, 289
Van Slyke, D. D., 5, 77, 78, 140, 150, 181, 273, 276
Van Straten, F. W., 15, 150
Vastagh, G., 77
Vaughn, T. H., 181
Verdino, A., 150, 209
Vetter, F., 100, 150
Viditz, F. von, 41, 262
Vieböck, F., 181, 209, 247, 251, 262
Vila, A., 204
Volhard, J., 181

Wagenaar, M., 294
Wagner, E. C., 77, 78
Wagner, R., 77
Waldschmidt-Leitz, E., 68
Wanscheidt, A., 150
Warburg, E., 15
Warburg, O., 276
Ware, G., 248, 251
Ware-Wellwood, G., 97, 99, 136, 150
Wasitzky, A., 294
Watts, C. E., 97, 100
Watzlaweck, O., 196, 197
Weatherill, P. F., 32
Weber, E., 60
Weil, H., 150
Weiss, F., 293
Werner, A., 177, 181, 198, 276
West, E. S., 78
West, W., 238
Westbrook, D. W., 41
Weston, P. E., 294
Weszelszky, J. V., 181
Weygand, C., 5, 41, 50, 95, 98, 100, 134, 150, 177, 181, 198, 276, 282, 294
Weyl, H., 271
White, E. V., 150, 251

White, E. P., 61
White, J. W., 295
Whitman, J. B., 50, 138, 148, 251
Wichmann, H., 180
Widmark, E. M. P., 61
Wiesenberger, E., 12, 15, 65
Willard, H. H., 32, 181
Willard, M. L., 292
Willenberg, H., 50, 134, 148
Williams, A. S., 276
Williams, P. A., 147
Williams, R. J., 32, 35, 209
Willstaedt, H., 276
Willstätter, R., 68, 251, 294
Wilson, C. L., 294
Wilson, C. W., 198
Wilson, D. T., 180
Wilson, D. W., 294
Winnacker, K., 146
Wintersteiner, O., 195, 198, 202, 204, 208, 209
Wirth, C., 181
Wise, L. E., 15, 145, 150
Wolff, H., 179
Wolfrom, M. L., 262
Wormley, T. G., 1, 5, 295
Wrede, F., 150, 208, 209
Wright, F. E., 294
Wright, G. F., 150, 181, 251
Wright, S. L., 215, 216
Wynne, A. M., 262

Yagoda, H., 61
Young, D. M., 99
Young, G. H., 198
Yuska, H., 78

Zacherl, M. K., 3, 5, 61, 149, 176, 181
Zakrzewski, Z., 61, 78
Zambotti, V., 204
Zappi, E. V., 150
Zechmeister, L., 295
Zeisel, S., 247, 250, 251
Zerewitinoff, T., 270, 272
Zetsche, F., 181
Zimmermann, W., 209
Zinn, J., 31
Zscheile, F. P., 295

SUBJECT INDEX

Absorption burets, 170, 177, 192
Absorption tubes, 111-114, 123-125, 136-137
  detrimental influences, 136
  electrostatic charges on, 125, 136
  filling of, 112, 113
  fork for, 124
  various types, 136-137, 207
Acetone, determination of, 273
Acetyl groups, determination of, 252, 257, 261
Acid halides, determination of, 264
Acids, determination of, 66-67, 263-264
Active hydrogen, determination of, 263-271
Acyl groups, determination of, 252, 232
Adsorption indicators, 176, 177, 195
Air column, 234
Air error, 94
Alcohols, determination of, 250, 262, 263
Aldehydes, determinations of, 263
Alkalimetric determinations, acyl groups, 252, 261
  carboxyl groups, 66-67
  halogen, 168, 176
  sulfur, 188, 195
Alkimide groups, determination of, 244-246, 249
Alkoxyl groups, determination of, 239, 247-248
Alkyl groups, determination of, 239-262, 274
  O-, 239-244, 247, 248
  N-, 244-246, 249
  S-, 249
Alkyl halides, determination of, 263
Aluminum, determination of, 65
Amides, determination of, 69, 263
Amines, determination of, 69, 263, 273
Amino acids, determination of, 68, 75, 263
Aminoid nitrogen, determination of, 69-77, 79-99
  acidimetric, 69-77
  gas-volumetric, 79-99
  iodometric, 76
  manometric, 77
  semi-micro, 76
  ultra-micro, 76
Ammonium phosphomolybdate, 199, 201-203
Analyses, application forms, 286
  records, 279, 280
  reports, 287
Analysis, organic, elementary, 62-209, 290
  qualitative, 290-291
  structural, 239-274
Analytical designing, 288
Anhydrone, 101, 121, 136, 266
  absorption tube, 112
Arsenic, determination of, 205-206
Asbestos, 120, 122, 134
  choking plug, 109, 134
  paper, 120
  platinized, 123
  shield, 119
Ascarite, 121, 136
  absorption tube, 113
Asepsis, chemical, 277, 281
Aspirator, 115, 125, 127
Aurates, determination of, 64
Azotometer, 85-88, 98, 99

Balances, 6-12
  microanalytical, 1, 2, 7-11, 15, 65, 283
    domestic, 6
    foreign, 1, 6, 8, 12
  ordinary analytical, 11-12
    calibration of, 11, 23-26
    selection of, 22
Barium, determination of, 64
Barium chloride, 185

Barium sulfate precipitate, 186, 193
  filtering of, 186-187, 193-195
  ignition of, 187, 194
  weighing of, 187, 194-195
Bath liquids, constants of, 300
Benzoyl groups, determination of, 252
Boats for weighing, 42
Boiling point, determination of, 226-227, 291
Bomb, for fusions, 165, 177, 193, 199, 205
  furnace, 165
  methods, for halogen, 165, 167, 177
    for phosphorus, 199, 201
    for sulfur, 193
  tubes, 151
Boric acid, standard solution, 76
Boron, determination of, 206
Bromine, 151, 160, 165, 168, 175, 178, 297
  determination of, 151-172
    alkalimetric, 168-172
    gravimetric, 151-167
      by dry combustion, 160-165
      by fusion, 165-167
      by wet combustion, 151-160
    with chlorine, 178
    with sulfur, 191, 197
Brush for balance, 10, 35
  camel's-hair, 35
Bubble counter, 104, 119, 134, 252
Burets, micro, 51-52, 58-59, 241
Burners, 36, 84, 106, 119

Cadmium, determination of, 64
Calcination method, 177
Calcium, determination of, 64
Calculations, 296-300
Camphor, 217, 299
Capillaries, for weighing, 45-46, 218, 225, 231
Capillary pipets, 38, 218
Caps, for absorption tubes, 114
  for filter tubes, 208
  for weighing tubes, 44, 49
Carbon, determination of, gravimetric, 101, 132
  manometric, 140-145
  titrimetric, 139-140
Carbon dioxide, gasometer, 82, 83
  generators, 81, 96, 97, 241
Carbon and hydrogen, determination of, 101-132, 139
  gravimetric, 101, 132
    accuracy in, 132
    combustion, proper, 128
    combustion train, 118
  faults, detection of, 127, 130, 135
    influence of other elements, 136
    in nitrogen atmosphere, 138
    oxygen control in, 126
    sample size limit, 145
    semi-micro, 145
    titrimetric, 139
    ultra-micro, 138-139
Carbonyl compounds, determination of, 263
Carboxyl compounds, determination of, 66-67, 263-264
Carius method, for halogen, 151, 160
  for sulfur, 182-188, 190
Catalytic combustion methods, 138, 160, 191, 273
  for carbon and hydrogen, 138
  for halogen, 160-165
  for sulfur, 188-190
Centrifuge cones, 39, 290
Centrifuges, 37, 231, 291
Cesium, determination of, 64
Chamois, 10, 35, 116
Chemicals, list of, 288-289
Chlorine, 151, 160, 165, 168, 175, 178, 297
  determination of, 151-172
    alkalimetric, 168-172
    gravimetric, 151-167
      by dry combustion, 160-165
      by fusion, 165-167
      by wet combustion, 151-160
    with bromine, 178
    with sulfur, 191, 197
Chromatographic analysis, 290
Chromium, determination of, 65
Cobalt, determination of, 65
Combustion furnaces, 106
Combustion methods for carbon and hydrogen determination, 101-132, 138-139, 145
  semi-micro, 145
  standard, 101-132
  ultra-micro, 138-139
  various others, 138-139

Combustion stand, 84
Combustion train, 118-121, 136
  all-glass, 136
Combustion tubes, for carbon and hydrogen determination, 107
    conditioning of, 110
    filling of, 107-110
  for Dumas method, 83
  for halogen and sulfur determination, 160, 184, 192
Conductometric methods, 273
Constants, for bath liquids, 300
  for solvents, 299
Copper, determination of, 65, 206
Copper oxide, 88, 122
Copper wool, 84, 89
Counterpoises, 30-31, 34
Crucibles, Gooch, 178
  Neubauer, 178, 194
  nickel, 193
  platinum, 194
  porcelain, 183-184
  silver, 199
Cryoscopic molecular-weight determination, 217-220
Crystallization procedures, 290
Cups for weighing, 44, 176, 177

Deflection sum, 16
Deflection unit, 15
Desiccator, 34, 43, 48
  vacuum, 36, 49, 199
Dichromate combustion methods, 139-145, 168-172, 176
Digestion flasks, Kjeldahl, 71, 72, 199
Digestion oven, 70, 199
Distillation procedures, 290
Double bonds, determination of, 273
Dropping bottles, 53
Drop-scale methods, 76, 203
Drying blocks, 36, 37, 49
Drying pistol, 48
Drying tubes, 114
Dumas nitrogen method, 79, 95

Ebullioscopic molecular-weight determination, 210-215
Electric furnaces, 105, 106
Electrometric methods, 273
Electrostatic charges, on absorption tubes, 116, 136
  in balances, 9
Equipment for students, 278
Esters, determination of, 239, 264
Ethers, determination of, 239-249
Ethoxyl groups, determination of, 239-244, 250
Ethyl alcohol, determination of, 239, 263
  in human brains, 250
Ethyl groups, determination of,
    O-, 239, 247
    N-, 244, 249
    S-, 249
Ethylene, determination of, 250
Evaporation apparatus, 40, 182-183
Explosive substances, combustion of, 138
  weighing of, 44
Extraction procedures, 290

Feather for precipitate transfers, 195
Files, 38
Fillings for combustion tube, in carbon and hydrogen determination, 107-108, 111, 134, 135
  in Dumas method, 83-84
Filter, dust, 189, 192
Filter-absorption tube, 138
Filter beaker, 178
Filter crucible, 195
Filter stick, 183
Filter tubes, 152-153, 178, 199, 208
Filtration apparatus, for halogen, 153-154
  for sulfur, 183-185, 186-187, 193-195
Filtration procedures, for ammonium phosphomolybdate, 202
  for barium sulfate, 186-187, 193-195
  for silver halide, 157-159
  general, 290
Flasks, Kells-Ringer, 225
  Mariotte, 114, 119, 120, 137
  suction, 153, 154, 184
  titration, 53, 241
  volumetric, 55, 231
Fluorine, determination of, 175
Forceps, 10, 33
Fork for weighing, 124

Furnace, electric, 105-106, 119, 120, 137
  for Carius method, 151, 182
  gas, 106, 119
Fusion method, for halogen, 165, 177
  for phosphorus, 201
  for sulfur, 193

Gamma gram, 6
Gasometer in Dumas method, 80, 82, 83, 97
Gasometric determination of nitrogen, 79-99
Generators, carbon dioxide, 96, 97
  methane, 264
Generic tests, 290
Glass bead in microburets, 51
Glass cement, 123
Glass cutting, 36, 38, 157
Glass thread, 231
Glass wool, 122, 231, 233
Gold, determination of, 64
Grignard reagent, 263, 266, 268
Group reactions, 263-264, 290

Halogen, determination of, 151-181
Halogen compounds, determination of, 263
Heating block, 169
Heating mortar, 106, 119, 137
Heating units, 105, 106, 119, 137
History of organic microanalysis, 1
Hydriodic acid, sp. gr. 1.7, 241, 249
  sp. gr. 1.96, 241
Hydrochloric acid solution, 0.01 *N*, 55
  0.1 *N*, 267
Hydrogen, active, determination of, 263-270
  determination of, gravimetric, 101-132
  ionic, determination of, 66-67
Hydrogenation methods, 177, 273
Hydroxyl groups, determination of, 262, 263
Hygroscopic substances, 45, 48, 49, 138

Immersion filter, 3, 183, 187
Indicators, 54, 75
Installation of microchemical laboratory, 283-289
Inverted filtration, 184, 186, 193
Iodine, determination of, 151-167, 172-175, 177-178
  gravimetric, 151-167
    by dry combustion, 160-165
    by fusion, 165-167
    by wet combustion, 151-160
  iodometric, 172-175
Iodine solution, 0.01 *N*, 257
Iodoform, determination of, 274
Iodometric determinations, acetone, 273
  acetyl groups, 257
  alkimide groups, 244, 246
  alkoxyl groups, 239, 243
  arsenic, 206
  iodine, 172-175
  sulfur, 195
  thiosulfate, 57
Ionic halogen, determination of, 178
Ionic hydrogen, determination of, 66-67
Iron, determination of, 65
Isolation procedures, 290
Isonitriles, determination of, 263
Isopropylidene groups, determination of, 273
Isothermic distillation, 230-238

Ketones, determination of, 263, 273
Kipp generators, 79-82, 96, 241
Kjeldahl apparatus, one-piece, 76
  usual, 70

Lead, determination of, 64
Lead peroxide, 122, 135
Liquid column, 234
Liquids, weighing of, 46, 48, 89, 218, 225
List of chemicals, 288-289
Lithium, determination of, 64

Magnesia reagent, 205
Magnesium, determination of, 64, 65
Magnesium perchlorate, 101, 121, 136, 266
Manganese, determination of, 64
Manometric combustion apparatus, 141
Marble, 88, 97
Mariotte flask, 114, 119, 120, 137
Measuring spoon, 167, 171
Measuring tube, 167

Melting point, determination of, 217, 219, 291
Melting-point apparatus, 217, 219
Mercury, determination of, 206
Mercury, for Dumas method, 89
  for vapor density method, 223, 300
Metals, determination of, 62-65
Methane, determination of, 263-271
Methoxyl groups, determination of, 239-244, 250
Methyl groups, determination of,
    C-, 274
    O-, 239, 247
    N-, 244, 249
    S-, 249
Methyl red, 54
Microanalysis, organic, history of, 1
    introduction in U.S.A., 2
    teaching of, 2, 277-282
Microbubbles, 82, 92, 95
  sticking of, 98
Microburets, 51-52, 58-59
Microburners, 36
Microchemical laboratory, installation of, 283-289
Microgram, 6
Micrometer, readings, 234
  scale, 230
Micromuffle, 62
Microscope, 230
Microscopical molecular-weight method, 237
Mixing tube, 89
Molecular refraction, determination of, 291
Molecular weight, determinations of, 210-237
Molybdate solution, 200
Molybdenum, determination of, 65
Mortar for heating, 106, 119. 137

Neubauer crucible, 178-194
Neutralization equivalent, 66-68, 297
Nickel, determination of, 65
Nickel catalyst, 207
Nickel crucible, 193
Nitric acid, concentrated, 152, 185
Nitric-sulfuric acid mixture, 200
Nitriles, determination of, 263
Nitro compounds, determination of, 263
Nitrogen, absolute, determination of, 79-99
  aminoid, determination of, 69-77
  Dumas method, 79-99
  for Grignard reaction, 265
  Kjeldahl method, 69-77
  reduction tables, 301-311
Nitrometer, 85-88, 98, 99
  calibration of, 87
  correction table, 88
Non-metals, determination of, 205, 209

Optical rotation, determination of, 274
Ordinal tests, 290
Organic analysis, qualitative, 290-291
Oxidation equivalent analysis, 207
Oxygen, determination of, 207, 208
Oxygen tank, 101, 133, 160, 168
Ozonalysis, 274

Pellet press, 37, 213
Perhydrol, 71, 161, 170, 188, 200, 201
Phenolphthalein, 54
Phenols, determination of, 263
Phosphorus, determination of, 199-204
Photoelectric methods, 273
Physical constants, determination of, 227, 274, 291
Pipets, precision, 38, 213
  sp. grav., 38
  weighing, 46, 224, 225
Platinates, determination of, 64
Platinized asbestos, 123
Platinum, determination of, 64
Platinum boat, 42
Platinum contacts, 160, 163
Platinum crucible, 194
Platinum cylinder, 63, 65
Platinum foil, 130
Platinum tetrahedrons, 210
Platinum wire, 34
Platinum wire gauze, 110, 116, 123, 136
Pointer, balance, 8, 11, 16
  scale, 15, 23
    division, 15, 23
    unit, 15, 23
Polarimeter tube, 274
Polarographic methods, 273
Porous tile, technic, 290
Potassium, determination of, 64

Potassium biiodate, preparation of, 51, 53
  solution, 0.01 *N*, 54, 71
Potentiometric methods, 273
Precipitation tubes, 161, 199
Precision, microanalytical balance, 17-20, 23-26
  ordinary analytical balance, 23
  sample relationship, 6
Precision pipets, 38, 72, 213
Preheater, 103, 118, 128, 133
Pressure regulator, 101, 118, 133
Pressure tubes for Carius method, 151, 155, 156
Proteins, determination of, 75
Purification procedures, 290

Qualitative organic analysis, 290-291
Quartz, platinized, 207
Quartz combustion tubes, 83, 107

Rack for tubes, 34
Rast molecular-weight method, 217-219
Reagents, list of, 288-289
Refractive index, determination of, 291
Residues, determination of, 62-64
Reversal, point of, in isothermic distillations, 236
    in weighing, 16
Reviews, first edition, 2
Rider, aluminum, 7, 8, 27, 30
  correct position of, 27
  quartz rod, 8, 31
Rider scale, 7, 11
  unit, 15, 23
Rubber connections, 117, 137
  impregnation of, 117
  lubrication of, 125
  marking of, 117
  sealing of, 81
Rubidium, determination of, 64

Safety tubes, 114
Sample, amount of, 6, 145, 154
  preparation of, 42-50
  weighing of, 23, 42-50
Schedules, teaching, 278, 281
Schlieren method, 291
Selenium, determination of, 208
Semi-micro combustion methods, 145
Semi-solids, weighing of, 45, 48, 89, 224
Sensibility of balance, 17
Sensitivity, of microanalytical balance, 17-20
    correction table, 21
  of ordinary analytical balance, 23-26
Silk thread, 115
Silver, determination of, 64, 208
Silver condenser, 70
Silver crucible, 199
Silver halide precipitate, 157-158
  dissolving of, 158
  drying of, 158
  filtering of, 157
  weighing of, 158
Silver wire, 122
Silver wool, 108, 122, 134, 173, 241
Siphon receiver, 241, 245
Sodium, determination of, 64
Sodium hydroxide on asbestos, 121, 136
Sodium hydroxide solution, 0.01 *N*, 56
  0.1 *N*, 203, 267
Sodium thiosulfate solution, 0.01 *N*, 56-57
Solids, weighing of, 42, 44, 45, 46, 48, 213, 218, 224
Solubility, determination of, 291
Solvents, constants of, 299
Spatula, 33
Specific gravity, determination of, 38, 291
Specific tests, 291
Spiral combustion tube, 160, 177, 184, 192
Standard solutions, 54-58, 231-232
  for isothermic distillation, 231-232
  hydrochloric acid, 0.01 *N*, 55
    0.1 *N*, 267
  iodine, 0.01 *N*, 257
  potassium biiodate, 0.01 *N*, 54
  sodium hydroxide, 0.01 *N*, 56, 71
    0.1 *N*, 203, 267
  sodium thiosulfate, 0.01 *N*, 56
Starch, 54, 242
Steaming apparatus, 53
Stopcock greases, 89, 137, 267, 297
Structure analysis, 239-274
Student, equipment, 279
  notebook, 279
  performance, 279-280

Sublimation procedures, 40, 290
Sulfur, determination of, 182-197
  alkalimetric, 188-190
  gravimetric, 182-188
    by dry combustion, 188-190, 191
    by fusion, 193
    by wet combustion, 182-188
  iodometric, 193, 195
  with halogen, 191, 197
Surface tension, determination of, 291
Syringe burets, 58

Tables, calibration nitrometer, 87
  carbon factors, 144
  logarithms, 312-330
  nitrogen reduction, 301-311
  precision-sample relationship, 6
  sensitivity correction, 21
Tares, bottles, 30, 34, 231
  metal, 29, 31
Teaching quantitative organic microanalysis, 2, 277-282
Teaching qualitative organic analysis, 290
Teaching schedules, 278, 281
Tests, generic, 290
  ordinal, 290
  specific, 291
Thermometers, Anschütz, 217
  Beckmann, 211, 212
Thiols, determination of, 263
Tiles, porous, technic of, 290
Tin, determination of, 65
Titration equipment, 51-53
Titration flasks, 53, 241
Titrations, acidimetric, 51, 69
  alkalimetric, 51, 66
  iodometric, 51, 57, 174, 195, 239, 243, 257, 260
  potentiometric, 261
Titrimetric determinations,
  of carbon, 139
  of carbon and hydrogen, 139
  of carboxyl, 66-67
  of Grignard reagent, 267
  of halogen, 168, 172, 176
  of phosphorus, 203
  of sulfur, 188, 195
Tongs for weighing, 31
Transesterification, 261
Transite plate, 85
Tubes, absorption, 111-114, 123-125, 136-137
  combustion, 83, 107, 160
  desiccator, 231
  filter, 152
  measuring, 167
  mixing, 90
  polarimeter, 274
  pressure, 151-152
  weighing, 44, 49

U-tube, 104, 117, 127, 134, 252
Unsaturation, determination of, 273
Urea, determination of, 69-74, 263, 273

Vacuum desiccators, 36, 49, 199
Vapor density determination, 221-229
Vaporimetric molecular weight determination, 221-229
Viscosity, determination of, 291
Viscous substances, weighing of, 45, 48, 89
Volhard titration method, 177
Volumetric flasks, 55, 231

Wash bottles, 39-40, 154, 192
Water, determination of, 101-132, 263, 271
Water absorption tube, 113
Waxes, weighing of, 45, 48, 89, 224
Weighing, directions for, 42
Weighing boats, 42, 43
Weighing bottles, 30, 45, 231
Weighing capillaries, 45, 46, 48
Weighing cups, 44, 176, 177
Weighing piggy, 49
Weighing pipets, 46, 47, 225
Weighing tube, 44, 49
Weights, calibration of, 28-30
  cleaning of, 30
Wet combustion methods, for carbon and hydrogen, 139-145
  for halogen, 151, 175, 176
  for phosphorus, 201
  for sulfur, 182-190

Zero point, balance, 16
Zero reading, 16, 17, 23
Zinc, determination of, 64

CENTRAL FOOD TECHNOLOGICAL RESEARCH INS
LIBRARY
* MYSOR

CHECKED
2008

VERIFIED
2013

CFTRI-MYSORE
450
Micro methods of..